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UNIVERSITY OF ALBERTA

EFFECTS OF STAND DENSITY ON ALFALFA (*Medicago sativa* L.) MORPHOLOGY, YIELD, AND QUALITY

BY

DOO-HONG MIN

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science.**

IN

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DEPARTMENT OF PLANT SCIENCE

Edmonton, Alberta

SPRING 1994

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **EFFECTS OF STAND DENSITY ON ALFALFA (*Medicago sativa* L.) MORPHOLOGY, YIELD, AND QUALITY** submitted by **DOO-HONG MIN** in partial fulfillment of the requirements for the degree of **Master of Science** in **Agronomy**.

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December 8th, 1993.

Abstract

The dehydrated alfalfa industry requires high quality plant material. To achieve this, dehydrated products are produced from alfalfa that is cut in the late bud to early flowering stage. The stands are often seeded at higher than normal rates and they may remain productive for only two to three years. The short stand life may be partly attributable to the high initial seeding rates which will reduce individual plant size and hence may reduce the plant's ability to survive the winter. The objectives of this study were: 1) to determine the optimum population density for a higher yield, better quality and longer persistence of alfalfa stands; 2) to compare gross morphology, forage quality, and winter survival of cultivars Algonquin and Vernal grown at a range of stand density; 3) to investigate the relationship among stand densities, individual plant size, carbohydrate storage, and winter survival of alfalfa.

To determine the optimum population density, alfalfa seedlings were transplanted at spacings of 6, 10, 15, 20 25 and 30 cm. Plants grown at the 4.5 cm plant spacing were directly seeded. Alfalfa stand density of alfalfa influenced shoot number per plant, yield per shoot and winter survival, and a combination of these three factors determined yield. The large alfalfa plants from low stand densities survived winter better than the small alfalfa plants from high stand densities under Edmonton environmental conditions. The amount of total nonstructural carbohydrates (TNC) per root increased and total etiolated growth (TEG) decreased as the stand density decreased. As stand density declined, the lethal temperature at which 50% of the plants were killed (LT 50) got lowered. Gross plant morphology was affected by plant spacing. Shoots per plant and yield per shoot increased linearly with increased plant spacing at all harvests. However, other morphological characteristics such as stem diameter, stem length, number of nodes per stem, leaf area per m^2, and leaf/stem ratio showed variable responses. Generally, crude protein (C.P.) increased with increasing stand density whereas acid detergent fiber (ADF) and neutral detergent fiber (NDF) were not affected by

the population density. However, there were cultivar differences; Algonquin had a higher C.P., a lower ADF and a lower NDF than Vernal.

High stand density appeared to be of little economic advantage in terms of winter survival, yield, persistence and forage quality.

It will be necessary to continue this study to ascertain the long-term effects of stand density on productivity, stand persistence, and forage quality.

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Chapter 1. Introduction.

Alfalfa (*Medicago sativa* L.) is an important agricultural crop in North America, where it is grown for forage, to enhance soil conservation, for green manure, and as a cash crop (Brenton et al., 1987). Alfalfa is called " the queen of the forages " because of its longevity, high yield, and high nutritive value (protein, energy, amino acids, calcium, phosphorous, carotene etc.). Stand persistence, high productivity and excellent forage quality are primary goals for most alfalfa producers.

The dehydrated alfalfa industry is one of the highest value adding agricultural industries on the Prairies and provides significant seasonal employment in rural areas. Alberta has natural advantages for the dehydrated industry because of its large forage production base, suitable climate, low cost of energy and extensive irrigation headworks (Anonymous, 1990). Alberta's dehydrated processing capability has been estimated at > 700,000 tonnes with 75 % of the product exported to Japan and other Pacific Rim countries (South-Korea, Taiwan, Hong Kong etc.). Generally, the dehydrated alfalfa industry requires high quality plant material with fine stems. Thus, dehydrated alfalfa pellets are processed from alfalfa cut in the late bud to early flowering stage (Anonymous, 1991). Higher (11.2 kg/ha) than normal seeding rates (9 kg/ha) are utilized and stands may remain productive for only two to three years (John, personal communication, October 5, 1993).

Competition can be defined as the struggle among individuals within a population for available resources, when the level of resources is below the combined needs of the numbers of the population. The resources involved in crop growth are primarily light, water, and nutrients (Harper, 1983).

Alfalfa can be described by three yield components: plants per area, shoots per plant, and yield per shoot. But the yield component concept is difficult to apply to seed-established swards of alfalfa because the number of plants per area is unknown unless roots and crowns are excavated, which results in destruction of the plot (Volenec et al., 1987). That's why transplanting of individual plants

into the field has been an effective method for establishing alfalfa at specific plant populations.

Ronningen and Hess (1955) reported that the yield per unit area in the third harvest year was significantly positively correlated with plant density, but not in the second year. Cowett and Sprague (1962) observed a decrease in both stems per plant and plant dry weight as populations increased from 11 to 86 plants m^{-2} whereas the yield per unit area increased. Rumbaugh (1963) also reported a decrease in both herbage yield per plant and stems per plant as population increased.

Several studies on alfalfa seeding rates indicated that seeding rates above 11.2 kg ha^{-1} did not significantly increase yield (Williams 1917, Willard et al. 1934, Carmer and Jackobs 1963, Zaleski, 1959). Zaleski (1959) obtained significantly lower alfalfa yields from a 5.6 kg ha^{-1} seeding rate compared to 11.2 kg ha^{-1}, but observed no significant difference in yield between seeding rates of 11.2 and 16.8 kg ha^{-1}. Carmer and Jacobs (1963) studied seeding rates of 4.5, 9.0, 13.4, and 17.9 kg ha^{-1} in 10.2 and 20.3 cm row spacing and broadcast seedings. They observed that total seasonal yields were higher when seeded at 9.0 kg ha^{-1} than at 4.5 kg ha^{-1}; but, no additional yield increase was obtained at rates higher than 9.0 kg ha^{-1}.

The plant population required for maximum herbage yield of alfalfa has been a subject of controversy, with estimates ranging from 22 to 215 plants m^{-2} (Tesar and Jackobs 1972, Bolger and Meyer 1983, Nelson et al. 1986). The ten-fold range in these estimates may be due, in part, to difficulty in accurately determining plant populations. Sund and Barrington (1976) excavated plants to determine the effects of seeding rate on plant population and herbage yield. Although plant populations in each of the three years following stand establishment (1969-1971) differed approximately three-fold, there was no effect on herbage yield. Jones et al. (1984) excavated alfalfa plants following termination of two variety trials to determine the relationship between plant population and herbage yield. In one trial, plant population was positively associated with herbage yield, while in the second trial, the nearly four-fold range in plant population (10 to 40 plants m^{-2}) had no effect on herbage yield.

The lack of a close, consistent association of plant population with herbage yield in this study and that of Sund and Barrington (1976) indicates that factors other than plant population may be more important determinants of yield of this species. The ability of surviving alfalfa plants to compensate for reductions in plant population by increasing other components (i.e., shoots per plant or yield per shoot) may represent a mechanism through which stand productivity is maintained. High seeding rates may increase production in the establishment year by providing more complete ground cover (Moline and Robinson, 1971), but the gaps tend to close up as time goes on (Kramer and Davis, 1949).

Density required for maximum yield varies depending on the area and climate, 140 plants m^{-2} in California (Marble and Peterson, 1981), 140 in Michigan (Tesar, 1977; 1978), 230 in Ohio (Van Keuren, 1973), and 260 in Illinois (Jackobs and Miller, 1970) produced maximum yields in the first harvest year after the seeding year.

As a stand aged fewer plants were necessary for high yields (Tesar, 1977: Tesar, 1979: Tesar and Marble, 1988). Tesar (1977) in Michigan, USA. found that yields in the first year after seeding increased as density increased to 140 plants m^{-2}. Seventh-year yields were 80% higher with 30 plants than with 10 plants m^{-2}. In the tenth year, Tesar (1979) reported a yield of 19.1 ton ha^{-1} with only 23 plants m^{-2}. According to Tesar and Marble (1988), approximately 150 to 250 plants m^{-2} were necessary for maximum yields in the year after seeding and 40 to 60 plants m^{-2} were adequate for maximum yields in older stands provided the stands were uniform. More recently, data from South Dakota (Twidwell and Kephart, 1991) suggest that 110-165 plants m^{-2} were adequate for maximum yield.

Several investigators have reported that forage yields in pure stands of alfalfa are not influenced by seeding rate above an adequate minimum seeding rate. However, this minimum rate varies among studies. Belzile and Rioux (1973), using seeding rates of 6.7, 13.4, and 20.2 kg ha^{-1}, obtained a 450 kg ha^{-1} yield increase for each additional 3 kg ha^{-1} seeds when the seeding was done early (28 April - 8 May), but when seeded at two later dates, there was no

difference in yield related to seeding rate. On the other hand, in Quebec, Genest (1973) found that two varieties of alfalfa seeded at 5.6, 11.2. 16.8, and 22.4 kg ha^{-1} exhibited significant increases in yield of both varieties from 5.6 to 11.2 kg ha^{-1} seeding rate, but very little increase in yield above the 11.2 kg ha^{-1} seeding rate for the variety Alfa, and a decreased yield for Vernal at the 16.8 kg ha^{-1} of seeding rate. A substantially higher yield of Vernal resulted from a 22.4 kg ha^{-1} seeding rate.

Roufail (1975) reported that Hunter River alfalfa (*Medicago sativa*) was sown at 6.7, 9, 13.4, 17.9, 22.4 or 26.9 kg ha^{-1}, broadcast or in 15, 30 or 45 cm rows, under irrigation, but seeding rate did not affect dry matter production in Victoria, Australia. Moline and Robinson (1971) found rates of 10 or 17 kg ha^{-1} to be superior in establishing stands of spring sown alfalfa on dry land. On the other hand, Jarvis (1962) found no significant difference between 43 and 1550 plants per m^{-2} on dry land. Under irrigation, seeding rates giving 50 to 200 germinating seeds m^{-1} of row had virtually no effect on yield (Bessac, 1968).

Previous studies cited have shown that as seeding rates increase the initial number of seedlings established increases. As time passes and some plants die, there can ultimately be equal plant densities for both low and high seeding rates (Carmer and Jackobs, 1963; Meyer, 1978; Sund and Barrington, 1976; Van Keuren, 1973). Carmer and Jackobs (1963) reported small plant size and lower survival rates as seeding rates increased. At the end of the year after seeding, they reported 80, 80, 73, and 64 percent survival in the 4.5, 9, 13.5, and 18 kg ha^{-1} seeding rates, respectively. Van Keuren (1973) reported a marked increase in plant numbers from high seeding rates compared with lower rates during the seeding year, but by spring of the following year the 13.5, 20.2, 27, 40.4, and 53.9 kg ha^{-1} had similar plant counts in North Dakota, USA. According to Bolger and Meyer (1983), percent plant mortality increased with plant density and losses were marked even in the low densities (i.e., 11 to 484 plants per m^{-2}). Twenty four to 36 % of the plants at the 22 to 43 plants m^{-2} densities and 10 % of the plants at the 11 plants per m^{-2} density failed to survive. In the alfalfa seed production research in

N.Z., the proportion of seeds producing alfalfa plants declined with seeding rates and, after establishment, deaths were density-dependent; higher seeding rates apparently did not improve persistence (Palmer and Wymn-Williams, 1976). Fribourg and Kennedy (1953) found a sharp reduction in alfalfa plant numbers during the first winter and a continued reduction in stand density through the second harvest year to a final count of 11 to 56 plants m^{-2} in the USA.

Work by Suzuki in P. E. I. (1991) found that there was a rapid reduction in plant numbers during the first year of a stand's life. At a seeding rate of 825 seeds per m^{-2} (approximately 18 kg ha^{-1}) more than 600 plants emerged but by the fall half of the plants had died and less than 100 survived the first winter.

Carbohydrates are the primary source of reserve energy stored in the vegetative organs of biennial and perennial forage plants. Stored food reserves in the roots and crown of alfalfa are an essential requirement for regrowth of shoots in the spring, initial regrowth after harvest, and for maintenance of crowns and roots during the winter (Smith, 1972). Knowledge of the accumulation and use of food reserves by perennial legumes is fundamental to an understanding of the influence of various cutting and fertility recommendations on the survival of alfalfa (Smith, 1962).

Of the four seasons, winter may be the most important in terms of alfalfa stand maintenance and long - term production. Winterkill is one of the most serious problems affecting alfalfa production in Western Canada. Work by McKenzie and McLean (1980a) found low fall carbohydrate reserves which resulted from harvesting during August or early September and low soil temperatures in December and January were two primary factors associated with winter injury. Jung and Smith (1960) observed that low food reserve levels limited the cold tolerance of alfalfa. Cold resistance was reduced during warm periods in winter (Suzuki, 1983) and the ability to reharden and maintain cold resistance with the onset of colder temperatures was favored by high food reserves in the overwintering part of the plant (Dexter, 1941). Alfalfa winter-hardiness in areas with severe winters traditionally has been predicted by fall growth score or

dormancy reaction (Smith, 1961; McKenzie et al, 1988; Stout and Hall, 1989). The fall dormancy reaction of very winter-hardy alfalfa cultivars to short days and cool temperature is characterized by prostrate or rosette herbage growth. In contrast, non-winterhardy cultivars produce tall, erect stems.

Field studies at the Beaverlodge Research Station, during the winter of 1975-1982, have suggested that three primary factors increase the potential for winterkill: 1) low food reserves in the roots and crowns, 2) water saturated soil conditions during the September and October hardening period, and 3) a late flush of growth in the fall from underground crown buds (McKenzie et al., 1983). According to Ouellet (1977), possibly the two most important factors causing winter injury are low air temperature and lack of snow cover, both of which influence soil temperature, and alfalfa stand loss is largely due to lethal soil temperature.

Low percentages of TNC in crowns and roots are common after repeated cuttings (Cooper and Watson, 1968). Reynolds (1971) noted relatively high TNC in alfalfa during early winter after frequent seasonal cutting, but stand density and yield had declined. But Jones et al. (1984) reported that total nonstructural carbohydrate was not correlated with stand density in Delaware, USA. Correlations were computed in an attempt to understand better relationships between parameters but coefficients were either not significant or were too low to identify relationships properly.

Work by Sund and Barrington (1976) found that the root % TNC among various rates of seeding (i.e., 6.7 - 40.4 kg ha^{-1}) in each of the sample years were quite similar, varying from a minimum of 25.6 in one set of samples to a maximum of 35.1 percent in another set of samples, but most of the readings were around 29 or 30 %. There was no consistent pattern in percent TNC according to seeding rates. However, when percent TNC was applied to root weights, the amount of TNC available for survival over winter was substantially higher in the roots from the 6.7 kg ha^{-1} of seeding rate because of their larger root size and declined progressively by rate to the lowest amount in the 40.4 kg ha^{-1} of seeding rate having the smallest roots.

Food reserves have also been indirectly measured by using etiolated growth (Klebasedel, 1971; McKenzie et al., 1988), in which the crown and root samples are collected from the field, and grown in the dark. Shoots are clipped every 2 week until their reserves are exhausted. It has been assumed that the growth of shoots in the dark, or total etiolated growth (TEG), is directly governed by the level of reserves in the crown and roots. McKenzie et al. (1988) reported that no significant correlation was observed between TNC and TEG method estimating stored food reserves in crowns and roots of alfalfa during the autumn and winter of 1981-1984, in Beaverlodge, Alberta. In an study in P.E.I., Suzuki (1991) reported that concentration of TNC in the crown and roots decreased slighly with age.

As a cold hardiness test, LT 50 is the lethal temperature at which 50 % of the population is killed. This method is a relative measure of the degree of temperature stress which the plants can tolerate. According to Suzuki (1991) young stands (2-4 yr of stand age) were slightly more cold hardy than mid-age stands (5-7 yr of stand age) in 1987, but the difference was insignificant in 1986 and no difference in LT50 was found between mid-age and old stands (8-10 yr of stand age).

At Charlottetown, Prince Edward Island, Suzuki (1972) stated that if the mean February soil temperatures droped below -4 °C, severe winterkill occured in all perennial legumes. This may be attributed to the influence of the wet Maritime climate on fall hardening since plants fail to harden properly under wet soil moisture regimes (Calder et al., 1965).

Under different plant populations shoot morphology can be influenced. Several studies were carried out to determine whether the morphological characteristics were changed by the plant population density or seeding rate. Recent studies in Indiana (Volenec et al., 1987) found that different population densities (i.e., 11, 22, 43, 97, and 172 plants m^{-2}) influenced shoot morphology. Averaged across cultivars (BIC, HI-PHY, and Vernal) yield per shoot, shoot number per plant, nodes per stem and stem diameter decreased as plant population increased. They also found that as

plant population densities increased, the direct effect of yield per shoot (YPS) became a more important determinant of yield per plant than shoots per plant. Mowat (1967) also reported that stems were thinner at the high density. According to Leach (1969) there were genetic differences in yield per shoot, and he observed a negative association between yield per shoot and shoots per plant. This suggests that dry matter partitioning to numerous shoots may be limited in genotypes which elongate shoots rapidly resulting in high yield per shoot (Volenec, 1985). Frakes et al (1961) reported that the direct effect of shoots per plant, of spaced-planted alfalfa, on yield was negligible, even though yield and stem number were highly correlated. Rumbaugh (1963) reported that the length of the longest stem of Ranger and Teton alfalfa varied with equidistant plant spacing of 13, 27, 53, or 107 cm, respectively. Stem length exhibited a curvilinear relationship with increasing plant spacing within varieties. Maximum stem length was attained at the 53 cm spacing (i.e., 4 plants m^{-2}) and declined thereafter (i.e., 27 or 13 cm plant spacing). As population density increased, crown width at soil surface and stem numbers per plant decreased.

Alfalfa stem diameter and branching was reduced in alfalfa stands established using high seeding rates (Hansen and Krueger, 1973; Krueger and Hansen, 1974; Radei, 1974). In a similar study, as plant density increased stems per plant decreased (Cowett and Sprague, 1963; Miller et al., 1969; Roufail, 1975). Cowett and Sprague (1963) found that stem numbers per plant decreased with increase an in stand density and that size of the crown at one harvest accounted for more than one-third of the number of stems per plant at the following harvest. They also reported that stem production per plant was not always associated with yield. Sund and Barrington (1976) also showed that a more dense stand had fewer stems per plant, with near equal numbers of stems per unit area for seeding rates of 6.7, 13.4, 20.2, 26.9, and 40.4 kg ha^{-1}.

Major factors that influence the potential feeding value of alfalfa during growth and development include: growth stage at the time of cutting: leaf to stem ratio: climatic and edaphic factors, such as geographic location, seasonal and yearly radiation, illuminance-

associated diurnal variation, ambient and soil temperature, soil type, soil moisture, and soil fertility, disease and insect damage and weed infestation, and growth stage at the time of cutting is a factor that determines the worth of alfalfa as a feedstuff (Marten et al., 1988). Acid detergent fiber (ADF) is composed of cellulose and lignin and is related to digestibility. Neutral detergent fiber (NDF) is composed of hemicellulose, cellulose, and lignin and is related to feed intake (Anonymous, 1990).

The influence of plant population on forage quality is not well researched. Bolger and Meyer (1983), using stands seeded in 1980, reported no effect of plant population (i.e., 11, 22, 33, 44, 55, or 100 plants m^{-2}) on concentrations of crude protein or ADF of alfalfa sampled in 1981. Meyer (1985) indicated that concentrations of ADF, acid detergent lignin (ADL), and NDF were unaffected by plant population even though shoot production ranged from 19 to 33 shoots per plant. According to Volenec et al. (1987) stems from plants grown at 172 plants m^{-2} populations contained 10 g kg^{-1} less lignin and were 30 g kg^{-1} more digestible than were stems from plants grown at 11 plants m^{-2} populations. They also found Vernal produced more and finer stems per plant, and these stems were lower in lignin and higher in digestibility when compared to cultivars BIC or HI-PHY. Buxton and Hornstein (1986) reported a negative association between *in vitro* dry matter digestibility (IVDMD) and lignin contrations in both alfalfa stems and herbage. Sund and Barrington (1976) also reported that seeding rates of 6.7 to 40.4 kg ha^{-1} did not influence cell wall constituents (CWC), ADF, or ADL in Wisconsin, USA. Van Keuren (1973) reported no effect of seeding rates (i.e., 3.4 - 54 kg ha^{-1}) on lignin, dry matter digestibility, or cellulose content in Ohio, USA. High digestibility of alfalfa has been reported to be related to a high leaf to stem ratio and low cell wall and lignin concentrations in herbage (Shenk and Elliot, 1970). Stem lignin concentration decreased and stem *in vitro* dry matter digestibility (IVDMD) increased with increased plant density (i.e., 11-178 plants m^{-2}). However, the effect of plant density on total forage IVDMD was less apparent, because leaf IVDMD concentrations were similar across plant densities (i.e., 80.8-81.3 % dry wt.). Stem NDF

and ADF concentrations followed a similar trend as lignin with plant density in Indiana, USA (Cherney et al, 1986). According to McGuire (1981) the crude protein content was not influenced by seeding rates of 8.4, 11.2, 16.9, or 22.5 kg ha^{-1} or row width of 6.7 or 13.4 cm in cultivars Vernal and DuPuits during 1963-1964 in Ohio, USA.

The objectives of this study were: 1) to determine the optimum stand density having high yield, forage quality and persistence for the dehydrated alfalfa industry; 2) to compare the gross morphology, forage quality, and winter survival of cultivars Algonquin and Vernal when grown at a range of stand densities; and 3) to investigate the interrelationships among stand density, individual plant size, carbohydrate storage, and the ability of alfalfa to overwinter.

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Chapter 2. Effects of stand density on yield, winter survival and morphology of Algonquin alfalfa.

2.1. Introduction

Alfalfa (*Medicago sativa* L.) yield can be described by the three yield components: plants per area, shoots per plant, and yield per shoot. The yield component concept is difficult to apply, however, to seed-established swards of alfalfa because the number of plants per area is unknown unless roots and crowns are excavated, and this results in destruction of the plot (Volenec et al., 1987). Thus, transplanting of individual plants into the field has been an effective method of establishing alfalfa at specific plant population densities.

The plant population required for maximum herbage yield of alfalfa has been a subject of controversy, with estimates ranging from 22 to 215 plants m^{-2} (Tesar and Jackobs 1972, Bolger and Meyer 1983). The ten-fold range in these estimates may be due, in part, to difficulty in accurately determining plant populations. Sund and Barrington (1976) excavated plants to determine the effects of seeding rate on plant population and herbage yield. Although plant populations in each of the three years following stand establishment differed approximately three-fold, there was no effect on herbage yield.

Density required for maximum yield in the first harvest year after the seeding year varies depending on the area and climate, 140 plants m^{-2} in California (Marble and Peterson, 1981), 140 plants m^{-2} in Michigan (Tesar, 1977; 1978), 230 plants m^{-2} in Ohio (Van Keuren, 1973), and 260 plants m^{-2} in Illinois (Jackobs and Miller, 1970).

As a stand aged fewer plants were necessary for high yields (Tesar, 1977: Tesar, 1979). Tesar (1977) in Michigan, USA. found that yields in the first year after seeding increased as density increased to 140 plants m^{-2}. Seventh-year yields were 80 % higher with 30 plants than with 10 plants m^{-2}. In the tenth year, Tesar (1979) reported a yield of 19.1 ton ha^{-1} with only 23 plants m^{-2}.

Previous studies have shown that as seeding rates increase the initial number of seedlings established increases, but plant survival

decreases, often resulting in ultimately equal plant densities for low and high seeding rates (Carmer and Jackobs, 1963; Van Keuren, 1973). In alfalfa seed production research in New Zealand, the proportion of seeds producing alfalfa plants declined with increasing seeding rates, and after establishment deaths were density-dependent; higher seeding rates did not improve persistence (Palmer and Wymn-Williams, 1976).

Work by Suzuki in P. E. I. (1991) found that there was a rapid reduction in plant numbers during the first year of a stand's life. At a seeding rate of 825 seeds per m^{-2} (approximately 18 kg ha^{-1}) more than 600 plants emerged but by the fall half of the plants had died, and less than 100 survived the first winter.

Several researchers have studied whether morphological characteristics can be changed by plant population density or seeding rate. Recent studies in Indiana (Volenec et al., 1987) found that different population densities (i.e., 11, 22, 43, 97, and 172 plants m^{-2}) influenced shoot morphology. Averaged across cultivars (BIC, HI-PHY, and Vernal) yield per shoot, shoot number per plant, nodes per stem and stem diameter decreased as plant population increased. They also found that as plant population densities increased, the direct effect of yield per shoot (YPS) became a more important determinant of yield per plant when compared to shoots per plant. Frakes et al (1961) reported that the direct effect of shoots per plant on yield of spaced-planted alfalfa was negligible, even though yield and stem number were highly correlated. Stem diameter and branching were decreased in alfalfa stands established using high seeding rates (Hansen and Krueger, 1973; Krueger and Hansen, 1974; Radei, 1974).

The objectives of this study were: 1) to determine the optimum population density giving high yield and persistence for the dehydrated alfalfa industry; 2) to investigate the influence of population density on winter survival; 3) to document morphological changes as influenced by plant population.

2.2. Materials and Methods.

The yield component concept is difficult to apply to seed-established swards of alfalfa because the number of plants per area is unknown, unless the roots and crowns are excavated, which results in destruction of the plot. Thus, to prevent destroying the plot and to know the exact stands per unit area, transplanting of individual plants into the field was carried out.

2.2.1. Plant material preparation in the greenhouse.

Root trainers were filled with a soil mixture composed of a 1:1:1 ratio of peat moss, black soil, and coarse sand. Approximately, 2000 (*Rhizobium meliloti* pre-inoculated) seeds of 'Algonquin' alfalfa (supplied by Prairie Seeds Inc.) were seeded into root trainers (1 x 1 x 4 inch, ' Fives ', Spencer-Lemaire Industries Ltd.) and grown for five weeks in a greenhouse at 20 C and 16 hours photoperiod. Plants were watered daily and fertilized bi-weekly with a liquid fertilizer ($N:P_2O_5:K_2O$, 20:20:20). The fungicide No-Damp was sprayed twice, on day one after planting and after emergence, in order to control any damping-off diseases that may attack the seedlings.

2.2.2. Transplanting in the field.

After five weeks growth in the greenhouse, seedlings were transplanted equidistantly into 1 x 1 m plots at distances of 6, 10, 15, 20, 25, and 30 cm plant spacing on July 3, 1991. These spacings represent populations of 278, 100, 45, 25, and 11 plants per m^2. Plants were irrigated after transplanting to facilitate establishment. The experimental design was a randomized complete block design with four replications.

2.2.3 Harvests for yield.

In the first production year of 1992 alfalfa plants, transplanted in 1991, were clipped by hand using scissors to a 5 cm stubble. Harvests occurred on June 18, July 27, and September 14, 1992, at which time plants were at one tenth bloom, one tenth bloom, and late bud stage, respectively.

In the second production year of 1993, plants were harvested at one tenth bloom, on June 7 and August 3, 1993. In order to determine yield per ha, 40, 15, 7, 4, 3, or 2 plants were clipped from the center area of 6, 10, 15, 20, 25, and 30 cm plant spacing plots, respectively. Harvested plant material was dried at 65C for 3 days in a forced air dryer, and then weighed.

2.2.4. Harvests for morphological characteristics.

In order to measure morphological characteristics (i.e., number of shoots per plant, yield per shoot, stem diameter, stem length, number of nodes per stem, leaf area per m^2, leaf to stem ratio) 7, 6, 5, 4, 2, and 2 plants from the plant spacings of 6, 10, 15, 20, 25, and 30 cm plots, respectively, were clipped by hand using scissors. The number of shoots per plant were counted. From these clipped plants, fifteen shoots were randomly selected, and yield per shoot was calculated by dividing the total dry weight of fifteen shoots (leaves plus stems) by fifteen.

With the 15 shoots selected, stem diameter at the lowest node, stem length, node number per shoot, leaf area, and leaf to stem ratio were measured. Leaves were separated from stems and their area was determined with a LI 3100 leaf area meter (Li-Cor Ltd., Lincoln, Nebraska). The leaf to stem ratio was calculated after drying the leaves and stems separately at 65C for 3 days.

2.2.5. Winter survival.

Live plants per plot within an area 50 cm by 2 rows were counted in mid September of 1991 and 1992, and again in late April of 1992 and 1993 in order to assess winter survival. The percent winter survival was calculated as:

% winter survival = (number of plants in the spring / number of plants in the fall) x 100.

2.2.6. Statistical analysis.

Data analysis was carried out by ANOVA procedures using GLM procedures of SAS (SAS Institute Inc., 1989). The plant spacing effects were partitioned into linear and quadratic components using orthogonal polynominal coefficients. The ANOVA tables can be found in the appendix.

2.2.7. Weather conditions.

The average monthly temperatures ((maximum + minimum) / 2) were recorded at the Edmonton International Airport by Environment Canada in Alberta from July, 1991 to August, 1993 (Appendix Fig. a.1). Generally, the highest air temperature occurred between mid-July and early-August. The air temperature of 1991 was a little higher than in 1992, 1993, or the 30 year average.

The monthly minimum temperatures during the period of early-November, 1991 to late-March, 1992 was on average warmer than the winter of 1992-1993, and warmer than the 30 year average (Appendix Fig. a.2). However, the monthly minimum temperature during the winter period of 1992-1993 was pretty close to normal.

The monthly maximum temperatures during the period of December, 1991 to April, 1992 were higher than the winter of 1992 to 1993, and higher than the 30 year average (Appendix Fig. a.3).

In terms of the total monthly precipitation during the growing

the season in the first production year 1992 it was drier than the 30 year average. Generally, it rained more in the second production year 1993 during the growing season than in the first production year 1992 (Appendix Fig. a.4).

2.3. Results

2.3.1. Yield

Alfalfa (*Medicago sativa* L.) was harvested three times during the first production year of 1992. In the first harvest, as plant spacing increased from 6 to 30 cm (278-11plants m^{-2}) there was no significant effect of plant spacing on dry matter yield. There was a significant ($p<0.01$) linear effect at the second and third harvest. The highest yield ha^{-1} was shown at the 30 cm plant spacing at the second and third harvest and the lowest yield ha^{-1} was attained at the 10 cm plant spacing with the second harvest, and at the 6 cm plant spacing with the third harvest.

In 1992, with each successive harvest the yield ha^{-1} decreased at each plant spacing and the difference in yield between plant spacings decreased at the second and third harvest (Fig.2.1). Annual total yield exhibited a significant ($p<0.001$) linear effect of plant spacing in the first production year. The annual total yield in 1992 was greatest for plants grown at the 30 cm plant spacing and least for plants grown at the 6 cm plant spacing (Fig.2.2).

The alfalfa plants transplanted in 1991 were harvested twice during the second production year, 1993. There was a significant ($p<0.05$) linear effect of plant spacing on annual total yield in the second production year (Fig.2.3). In the second production year, the difference in annual total yield decreased between high and low population densities. The yield of the second harvest was a little higher than that of the first harvest.

Total annual yield was higher in the first production year than that of the second production year

2.3.2 Winter survival

To measure the percent of winter survival, plant numbers per unit area were counted in the spring of 1992 and 1993. There was a significant linear ($p<0.001$) and quadratic ($p<0.001$) effect of plant

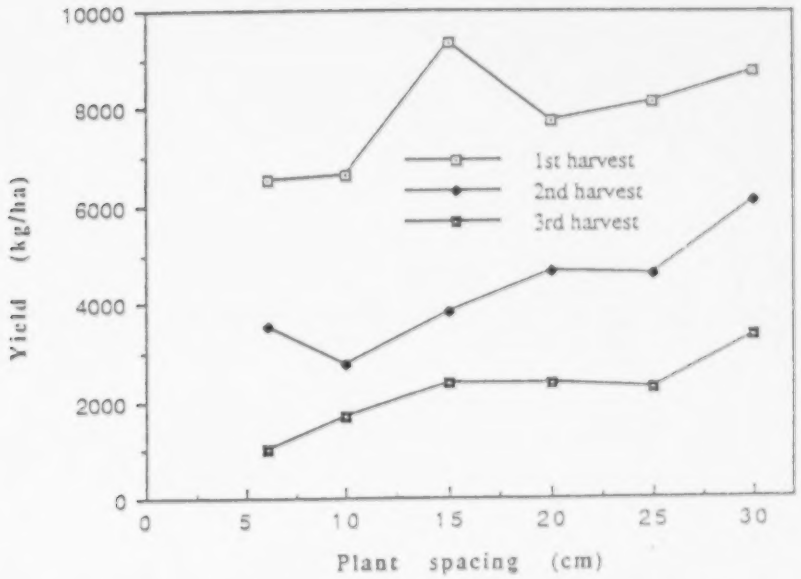

Figure 2.1. Effect of plant spacing on yield in the first, second, or third harvest of 1992.

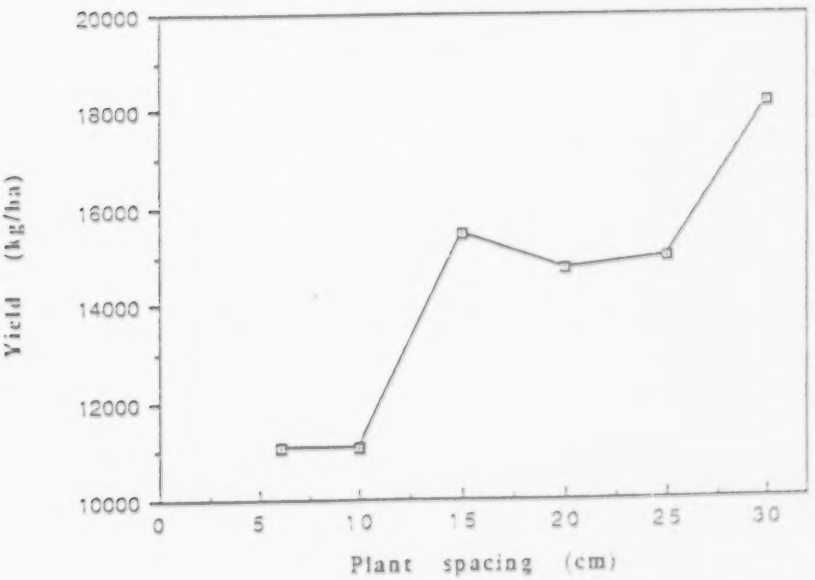

Figure 2.2. Effect of plant spacing on annual total yield in the first production year of 1992.

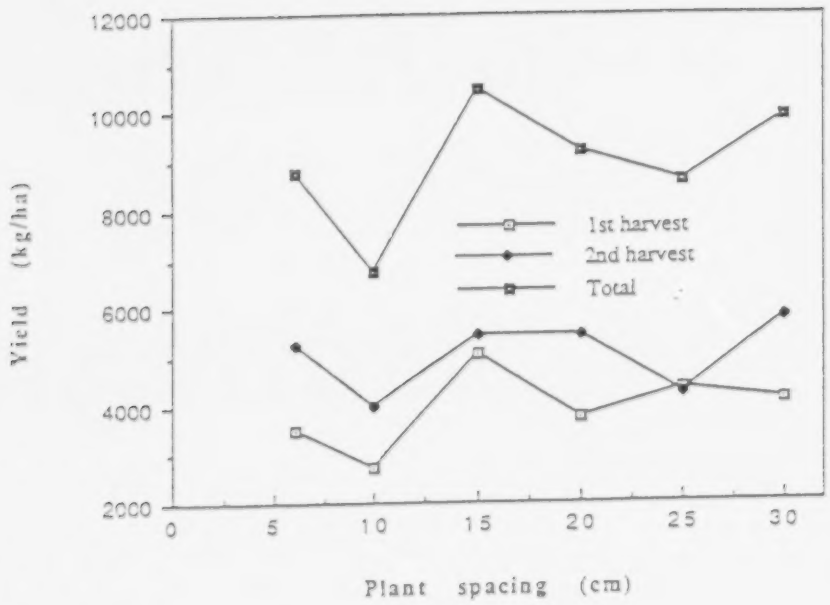

Figure 2.3. Effect of plant spacing on yield at the first, second harvest, or annual total yield in the second production year of 1993.

spacing on survival during the winter of 1991 - 1992. As shown in Fig.4, winter survival was lower at high population densities (i.e., 100 or 278 plants m^{-2}) than at lower population densities (i.e., 11, 16, or 25 plants m^{-2}). The lowest level of winter survival occurred at the 6 or 10 cm plant spacings (corresponding to 278 or 100 plants m^{-2}, respectively). The greatest decline in winter survival occurred between the 10 and 15 cm plant spacing. At the 20, 25, or 30 cm plant spacing (corresponding to 25, 16, or 11 plants m^{-2}, respectively) the winter survival was 100 %.

There was a significant linear ($p<0.001$) and quadratic ($p<0.001$) effect of plant spacing on survival during the winter 1992 - 1993. The winter survival in this year was higher compared to that of the previous year, even at the higher population densities (i.e., 278 or 100 plants m^{-2}) where winter survival was 86.9 and 90.2 %, respectively There were no dead plants at the 15, 20, 25, or 30 cm plant spacing.

2.3.3. Morphology.

2.3.3.1. Shoots per plant in the spring of the first and second production year.

After the first winter (1991 - 1992) there was a significant ($p<0.001$) linear effect of plant spacing on spring shoot number per plant. As plant spacing increased shoots per plant in the spring increased linearly (Fig.2.5). Plants grown at a 6 cm plant spacing produced 6.8 shoots per plant whereas plants grown at a 30 cm plant spacing attained an average of 26.3 shoots per plant. This equates to a 3.9 fold increase in shoots per plant as compared with 6 cm plant spacing. The trend for spring shoots per plant in 1993 was similar to that of 1992; as plant spacing increased the shoots per plant increased. Again, there was a significant ($p<0.001$) linear effect of plant spacing and the spring shoot number per plant in 1993 was slightly higher than 1992.

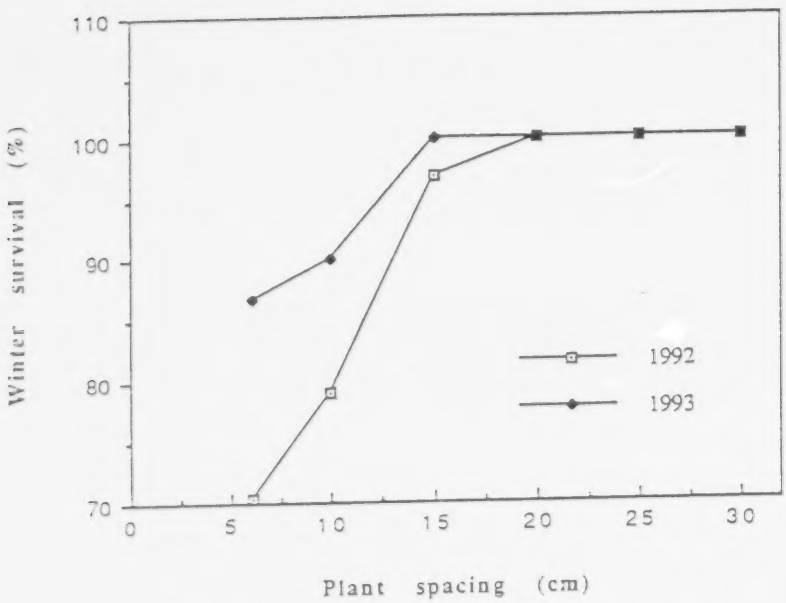

Figure 2.4. Effect of plant spacing on winter survival in the spring of 1992 and 1993.

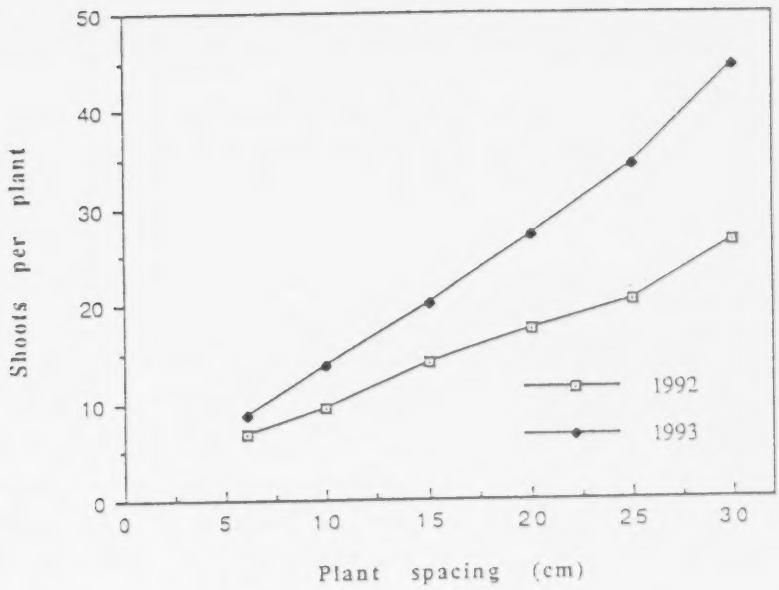

Figure 2.5. Effect of plant spacing on shoots per plant in the spring of 1992 and 1993.

2.3.3.2. Shoots per plant at harvest

As plant spacing increased the number of shoots per plant increased, at each harvest in the first production year of 1992 (Fig.2.6). At the first harvest of 1992 there was a significant ($p<0.001$) linear and ($p<0.05$) quadratic effect of plant spacing on shoots per plant. Plants grown at the 6 cm plant spacing had the lowest number of shoots per plant (6.8) whereas plants grown at a 30 cm plant spacing showed the highest shoots per plant (44.4). The greatest increase in shoots per plant occurred between the 25 and 30 cm plant spacing. The trends for shoots per plant at the second and third harvests in 1992 were quite similar to those observed at the first harvest. With successive harvests the difference in shoots per plant between the 6 and 30 cm plant spacing increased from 6.5, to 7.6 and 8.7 fold in the second, and third harvests, respectively.

There was a significant ($p<0.001$) linear effect of plant spacing on shoots per plant in the first and second harvests in 1993 (Fig.2.7). This is similar to the trend observed in 1992. As plant spacing increased the shoots per plant increased. With successive harvests there was little change in shoot number per plant.

2.3.3.3. Yield per shoot

As plant spacing increased, yield per shoot increased significantly in a linear ($p<0.001$) relationship at each harvest in 1992 (Fig.2.8). The yield per shoot of plants grown at a 6 cm plant spacing were the lowest at 0.36, 0.2 and 0.11 g at the first, second and third harvests, respectively. Plants grown at a 30 cm plant spacing showed the highest yield per shoot at 2.1, 0.85 and 0.56 g at the first, second and third harvests. However, with successive harvests the yield per shoot generally decreased across all plant spacings.

In the second production year, 1993, the yield per shoot at the first harvest, showed a significant ($p<0.001$) linear increase as plant

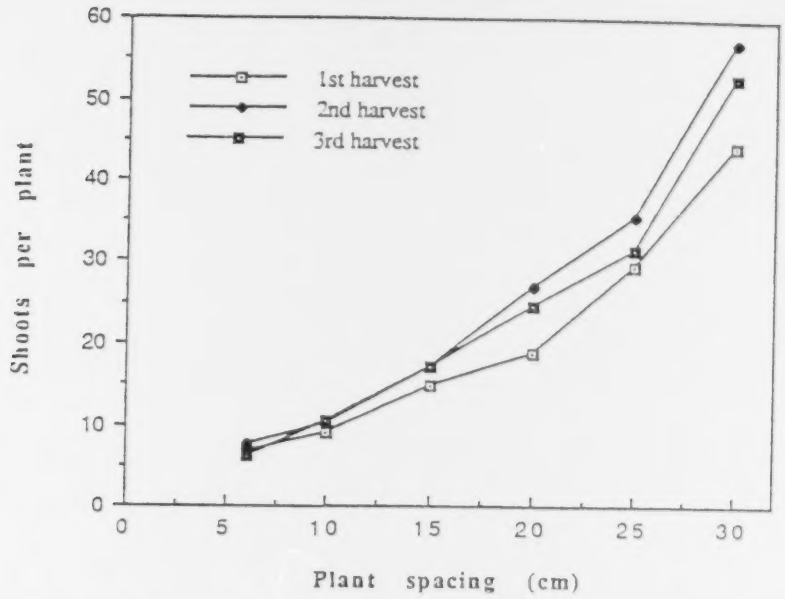

Figure 2.6. Effect of plant spacing on shoots per plant at the first, second and third harvest in the first production year of 1992.

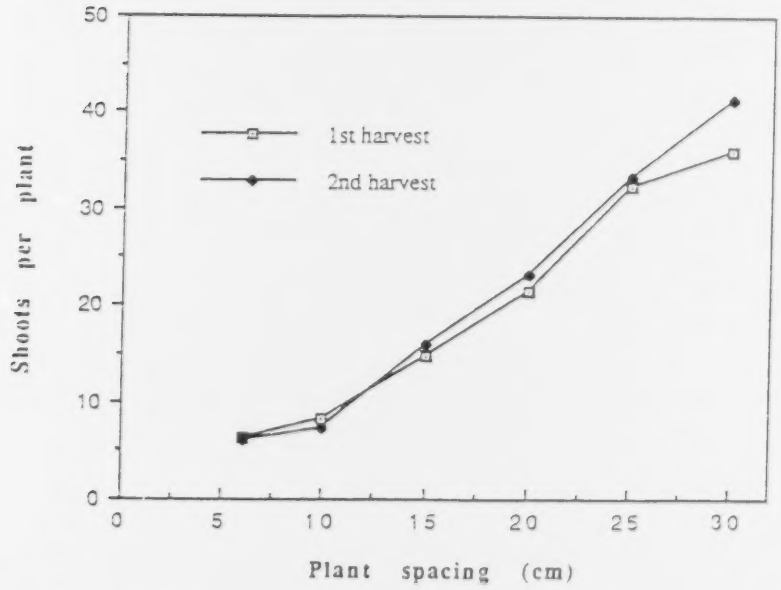

Figure 2.7. Effect of plant spacing on shoots per plant at the first and second harvest in the second production year of 1993.

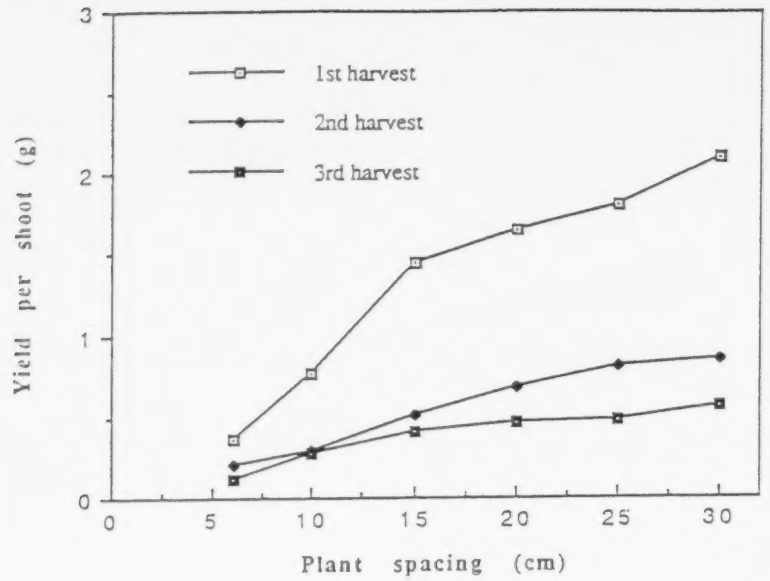

Figure 2.8. Effect of plant spacing on yield per shoot at the first, second and third harvest in the first production year of 1992.

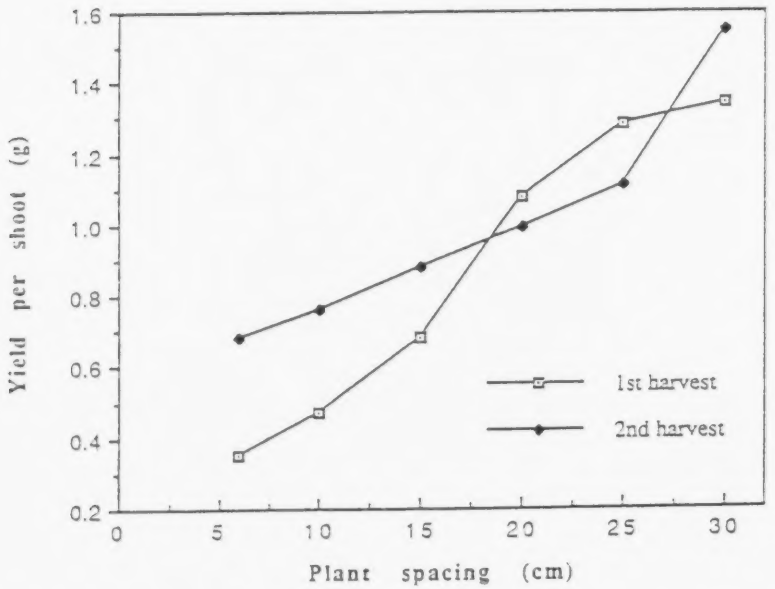

Figure 2.9. Effect of plant spacing on yield per shoot at the first and second harvest in the second production year of 1993.

spacing increased (Fig.2.9). At the second harvest of 1993 the yield per shoot increased as plant spacing increased in a significant ($p<0.001$) linear and ($p<0.01$) quadratic relationship with plant spacing.

2.3.3.4. Stem diameter

In the first production year of 1992 the stem diameter increased as plant spacing increased in a significant ($p<0.001$) linear relationship with plant spacing at the first and second harvest. At the third harvest there was a significant ($p<0.001$) linear and ($p<0.01$) quadratic relationship between plant spacing and stem diameter (Fig. 2.10). At each harvest in 1992, plants grown at the 6 cm plant spacing had stems with the smallest diameter. With successive harvests the stem diameter decreased at all plant spacings. As shown in Fig. 10, there was little difference in stem diameter between the 10 to 25 cm plant spacing in the second or third harvest of 1992, respectively.

In the second production year, as plant spacing increased, stem diameter increased (Fig.2.11). There was a significant ($p<0.001$) linear effect of plant spacing on stem diameter at each harvest. The smallest stem diameter was attained from plants grown at the 6 cm plant spacing and greatest for 30 cm plant spacing in each harvest. There was little change in stem diameter between the first and the second harvest in 1993.

2.3.3.5. Stem length

Generally, as plant spacing increased, the stem length increased (Fig.2.12). At the first harvest in the first production year of 1992, stem length increased as plant spacing increased in a significant ($p<0.01$) linear and ($p<0.05$) quadratic relationship with plant spacing. At the second harvest, there was a significant ($p<0.05$) linear relationship with plant spacing. At the third harvest there was a

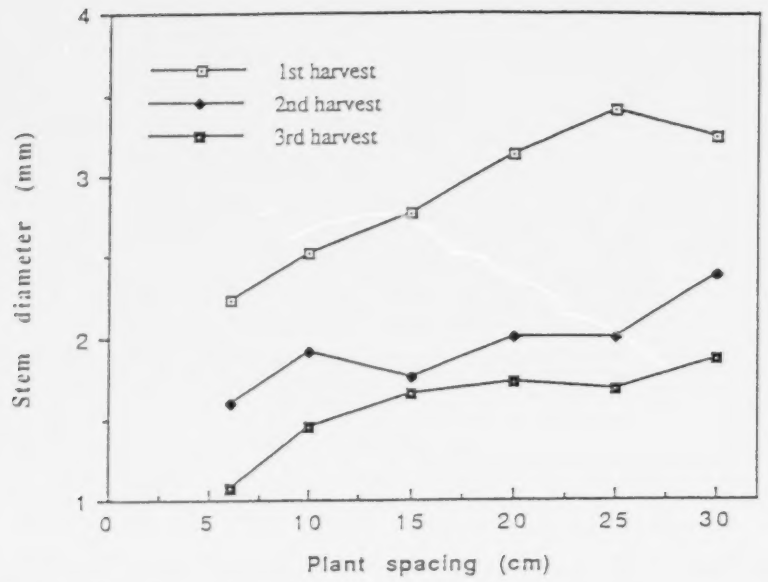

Figure 2.10. Effect of plant spacing on stem diameter at the first, second and third harvest in the first production year of 1992.

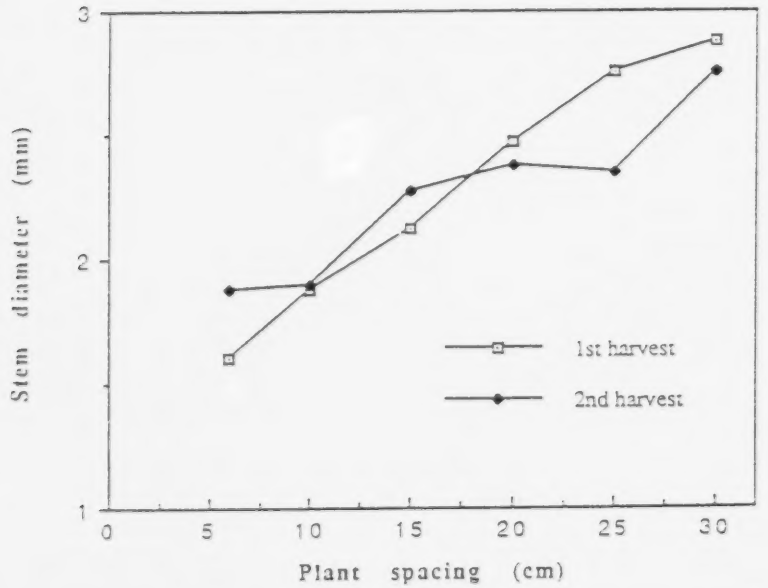

Figure 2.11. Effect of plant spacing on stem diameter at the first and second harvest in the second production year of 1993.

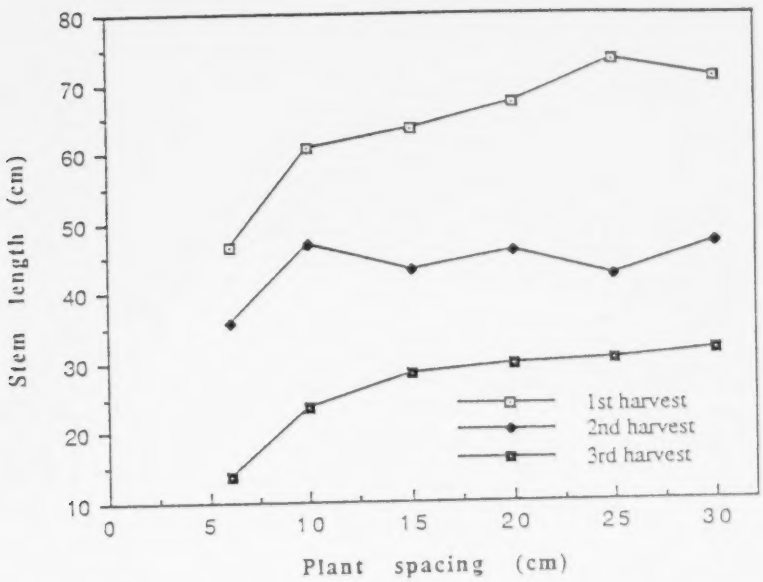

Figure 2.12. Effect of plant spacing on stem length at the first, second and third harvest in the first production year of 1992.

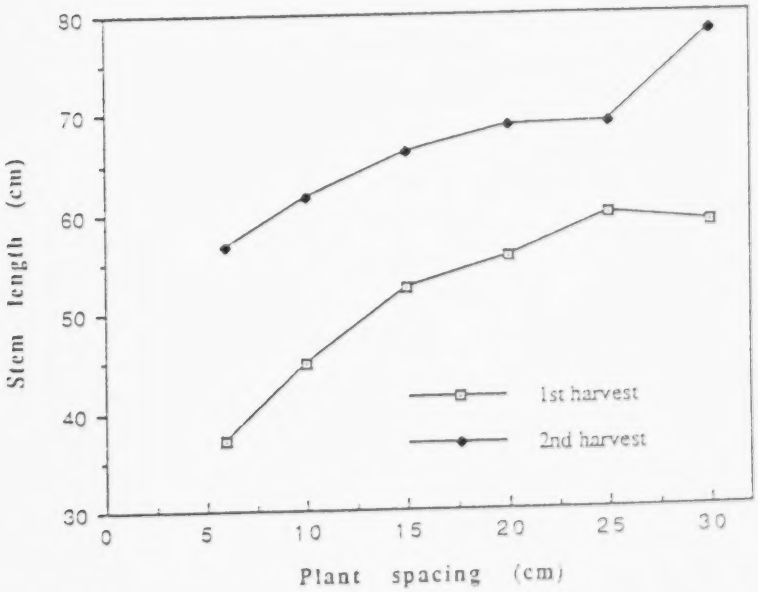

Figure 2.13. Effect of plant spacing on stem length at the first and second harvest in the second production year of 1993.

significant ($p<0.001$) linear and ($p<0.001$) quadratic relationship with plant spacing. With successive harvests the stem length greatly decreased at all plant spacings.

In the second production year 1993, as plant spacing increased the stem length increased (Fig.2.13). There was a significant ($p<0.01$) linear and ($p<0.01$) quadratic relationship between plant spacing and stem length at the first harvest. There was very little difference in the stem length between the 25 and 30 cm plant spacing at the first harvest. At the second harvest, there was a significant ($p<0.01$) linear effect of plant spacing on stem length. The stem length was greater at the second harvest than the first harvest.

2.3.3.6. Nodes per stem

In the first harvest of the first production year of 1992, the number of nodes per stem increased as plant spacing increased in a significant ($p<0.001$) linear and ($p<0.01$) quadratic relationship with plant spacing (Fig.2.14). However, the number of nodes per stem was not affected by the plant spacing at the second harvest. At the third harvest there was a significant ($p<0.01$) linear relationship between plant spacing and the number of nodes per stem. At each harvest, the 6 cm plant spacing had the lowest number of nodes per stem.

In the second production year of 1993, as plant spacing increased, the number of nodes per stem increased. At the first and second harvests there was a significant ($p<0.01$) linear relationship between plant spacing and nodes per stem (Fig.2.15). At each harvest the number of nodes per stem was lowest for plants grown at a 6 cm plant spacing. It was greatest for plants grown at the 30 cm plant spacing at the first harvest, and the 20 cm plant spacing at the second harvest. The number of nodes per stem at the second harvest was greater than at the first harvest at all plant spacings except at a 20 cm plant spacing.

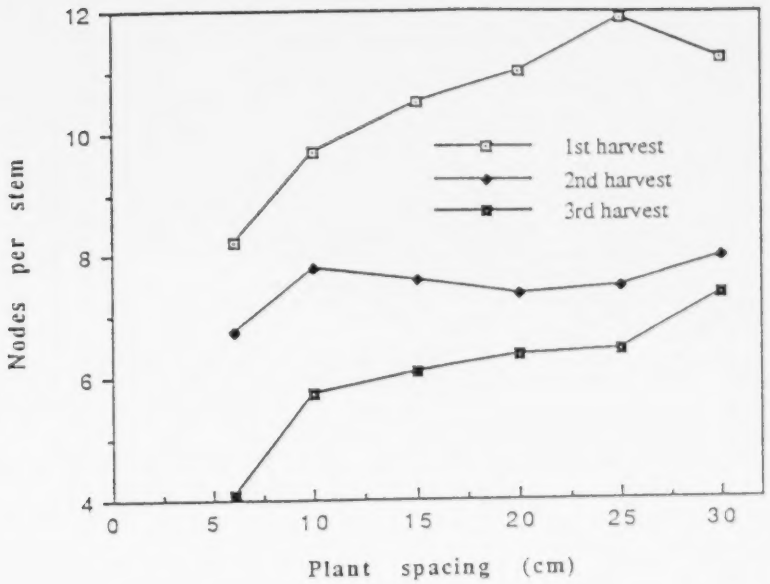

Figure 2.14. Effect of plant spacing on nodes per stem at the first, second and third harvest in the first production year of 1992.

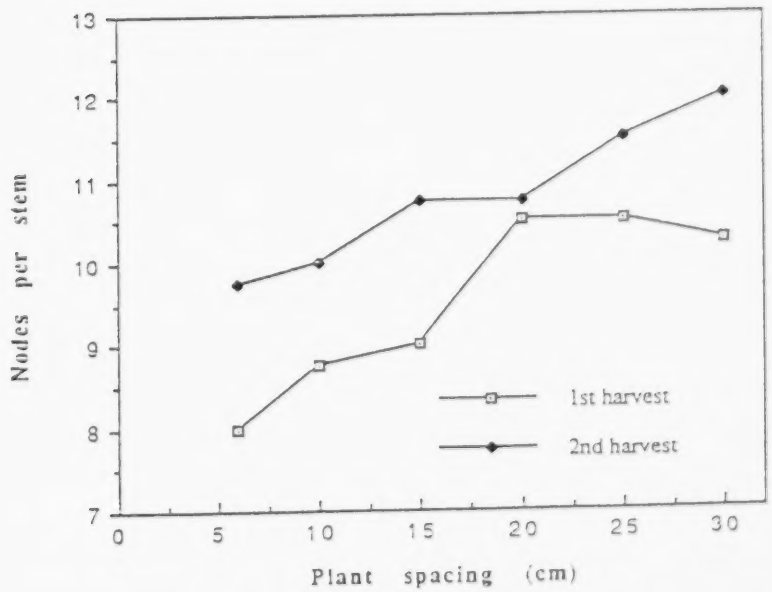

Figure 2.15. Effect of plant spacing on nodes per stem at the first and second harvest in the second production year of 1993.

2.3.3.7. Leaf area per m^2.

There was a significant ($p<0.001$) linear and ($p<0.001$) quadratic effect of plant spacing on leaf area per m^2 at the first harvest in the first production year of 1992. Leaf area per m^2 decreased from the 6 cm to 20 cm plant spacing and then increased at a 30 cm plant spacing (Fig.2.16). The greatest leaf area per m^2 occurred at the 6 cm plant spacing and the least for the plants grown at the 20 cm plant spacing. At the second and third harvest there was no significant difference in leaf area per m^2 between plant spacings. With successive harvests the leaf area per m^2 decreased at all plant spacings.

In the second production year of 1993, there was no significant effect of plant spacing on the leaf area per m^2 at the first and the second harvest (Fig.2.17). The leaf area per m^2 of the second harvest was a little higher than that of the first harvest.

2.3.3.8. Leaf to stem ratio

At the first harvest in the first production year of 1992, there was no significant effect of plant spacing on leaf to stem ratio (Fig.2.18). At the second harvest there was a significant ($p<0.05$) linear and ($p<0.05$) quadratic relationship between plant spacing and leaf to stem ratio, however there was very little difference in this ratio between the 10 and 30 cm plant spacing. At the third harvest there was a significant ($p<0.001$) linear and ($p<0.01$) quadratic relationship between plant spacing and leaf to stem ratio. With successive harvests leaf to stem ratio increased at all plant spacings.

In the second production year of 1993, there was a significant ($p<0.05$) linear and ($p<0.01$) quadratic effect of plant spacing on the leaf to stem ratio at the first harvest. There was also a significant ($p<0.05$) linear and ($p<0.05$) quadratic relationship between plant spacing and leaf to stem ratio at the second harvest (Fig.2.19). The greatest leaf to stem ratio occurred at the 6 cm plant spacing at the first harvest and at the 30 cm plant spacing at the second harvest.

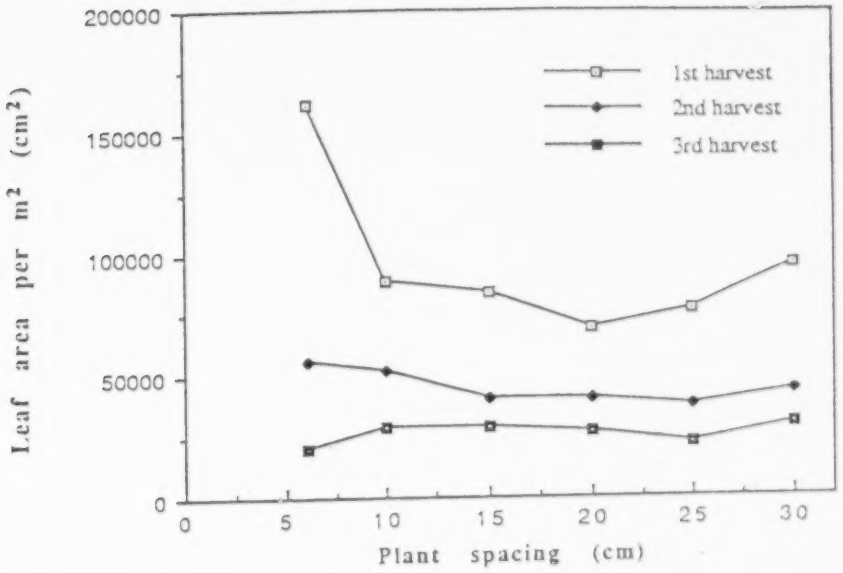

Figure 2.16. Effect of plant spacing on leaf area per m^2 at the first, second and third harvest in the first production year of 1992.

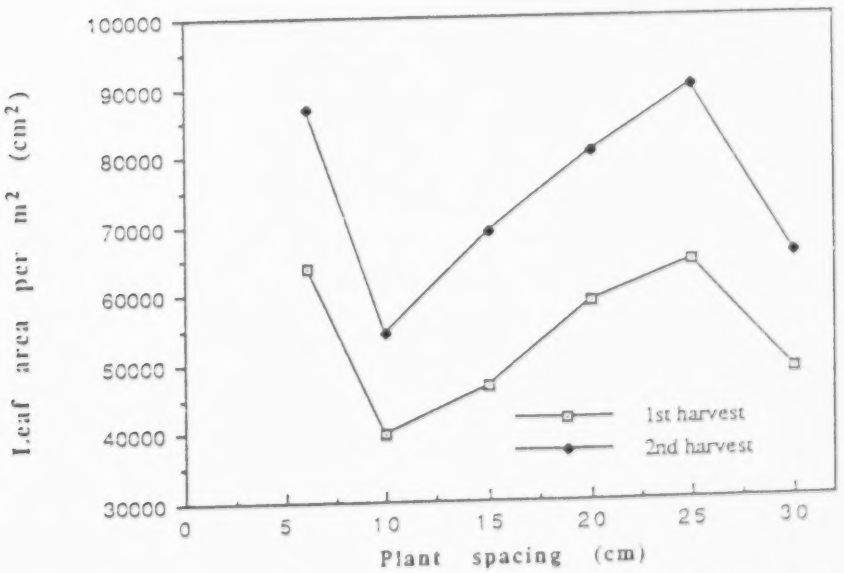

Figure 2.17. Effect of plant spacing on leaf area per m^2 at the first and second harvest in the second production year of 1993.

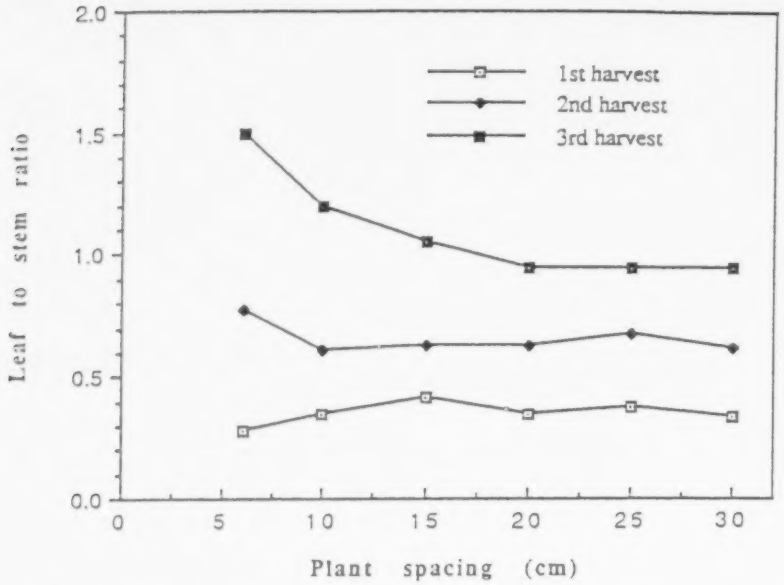

Figure 2.18. Effect of plant spacing on leaf to stem ratio at the first, second and third harvest in the first production year of 1992.

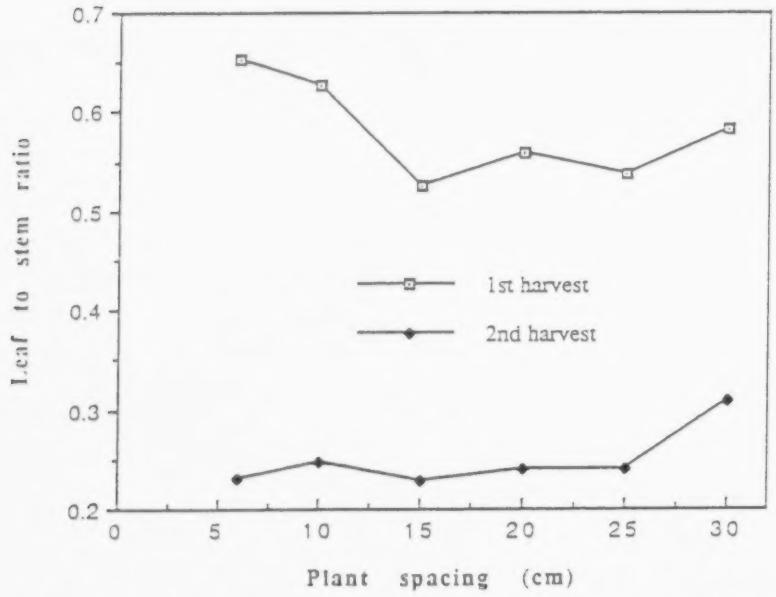

Figure 2.19. Effect of plant spacing on leaf to stem ratio at the first and second harvest in the second production year of 1993.

The lowest leaf to stem ratio occurred at the 15 cm plant spacing at each harvest. With successive harvests the leaf to stem ratio declined.

2.4. Discussion.

Stand density is one of the factors that determines yield and persistence of alfalfa. However, the optimum stand density varies with climate and soil conditions. As establishment costs increase, the question arises whether present seeding rates or population densities are optimum for obtaining maximum yield, quality and persistence.

Generally, alfalfa yield can be described by three yield components: plants per area, shoots per plant, and yield per shoot (Volenec et al., 1987). The lower yield at the high population densities in the first production year could be attributed to lower winter survival (Fig.2.4) during the winter period of 1991-1992, fewer shoots per plant (Fig.2.6), and lower yield per shoot (Fig.2.8) than at the low population densities. However, the decreased difference in annual total yield between the high and low population density in the second production year was partly explained by: i) smaller differences in average yield per shoot ((1st harvest + 2nd harvest)/2) between high and low stand density in the second production year than the first production year, ii) decreased shoot number per plant at the low stand density and no change at the high population density in the second production year compared with the first production year, iii) even though some plants died at the high population density during the first winter period of 1991-1992, there were still more plants per unit area than at the low population density. Thus, as plants aged from year to year, the yield difference between high and low population densities decreased. However, it is not apparent from this study for how long the differences in yield between high and low stand densities will persist.

Plant number per unit area changed over time as a result of differential winter survival. After the establishment year, the highest winterkill occurred at a 6 cm plant spacing. Although the mean winter temperature in 1991-1992 was not as low as that of 1992-1993 (Fig.a1), more plants were killed at the low population densities during the first winter. The decreased winterkill during the second winter in the high population densities might be attributable

to less intra-specific competition for resources as a result of reduced stand densities compared to the first winter. Those plants that survived the first winter may have had a greater genetic potential to winter harden. At the lower population densities, the alfalfa plants were larger and had low levels of winterkill during the first winter compared to the plants at the high population densities. There was very little or no winterkill in the second winter (Fig.2.4). The ability of larger alfalfa plants, at lower population densities, to survive during the winter may be attributed to a larger root and crown system and more extensive carbohydrate and nitrogen storage reserves. In this study, it is unclear which was the major factor (i.e., low soil temperature, lack of snow cover, snow mold pathogens, low carbohydrate reserves and tap root protein, ice encasement or frost heave) causing winterkill at the 6 and 10 cm plant spacing during the first winter. In Indiana, where alfalfa plants were established at populations of 11 and 172 plants m^{-2}, 90% of the plants survived the first winter (Volenec et al., 1987). These different results indicate that the extent of winterkill is strongly influenced by geographic region and climate.

The lower shoot number and yield per shoot at the higher population densities than at the lower population densities might be partly attributable to plant density effects on root and crown size, resulting from intra-specific competition for light, moisture, or nutrients. These findings are consistent with previous work (Bolger and Meyer, 1983; Volenec et al., 1987). As well, the limited availability of resources presumably results in less biomass production for each plant because of lower levels of photosynthesis.

It was hypothesized that when some plants in a population die, the surviving plants will have access to more resources. In this study, however, despite the death of around 20-30% of the plants at the high population densities, the surviving plants did not increase their shoot number per plant and the average yield per shoot increased only marginally in the second production year.

In this study, in the first and second production year, more shoots per plant and a higher yield per shoot at the lower population densities compensated for lower plant numbers. At the high

population densities even though there were more plants per unit area, lower winter survival, lower shoot number per plant and lower yield per shoot resulted in lower yields.

Generally, stem diameter increased as population densities decreased. At a high population density the basal leaves of alfalfa are heavily shaded resulting in a lower photosynthetic capability and a smaller shoot biomass than at a low population density. As well, at a high plant density each plant presumably will have less opportunity to enlarge their crown area from which the crown buds develop, due to lack of sufficient space. These findings agree with results from previous work (McGuire 1981; Volenec et al 1987).

Trends in stem diameter, stem length, and nodes per stem, leaf area per m^{-2}, and L/S ratio did not follow the yield trends at each harvest. This is reflected in the very low correlations between yield and morphological traits, and indicates that yield was affected very little by these morphological characteristics.

In this study, high population densities (100 and 278 plants m^{-2}) appeared to be of little advantage in terms of winter survival, yield, and persistence compared to low densities (45 plants m^{-2} or less).

2.5. Conclusions.

In the first and second production year, plants grown at the high population density had lower total annual yield due to lower winter survival, fewer shoots per plant and lower yield per shoot than at the low population densities.

In the second production year, yield differences between high and low plant densities declined compared to the first production year.

Alfalfa plants at a high population density were smaller and had lower levels of winter survival than the larger plants at a low population density. Generally, more winterkill occurred during the first winter than the second winter.

The shoot number per plant, yield per shoot, and stem diameter increased linearly with decreasing population densities. However, the other morphological characteristics, stem length, node number per stem, leaf area m^{-2}, and leaf to stem ratio showed variable responses to plant density.

From this study, a high population density appeared to be of little economic advantage compared to low stand density in terms of winter survival, yield and persistence.

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Chapter 3. Effects of stand density on alfalfa yield, winter survival, cold hardiness, morphology and quality in the cultivars Algonquin and Vernal.

3.1. Introduction

The relationships among yield, quality and persistence in relation to stand age of alfalfa are not predictable because of complex and often transitory interactions of genotypes, environment, and management. However, determination of alfalfa stand density and yield relationships is very important for the producers because this relationship, coupled with economics, determines when farmers should plow down stands and when stands should be kept.

Generally, alfalfa yield can be described by three yield components: plants per area, shoots per plant, and yield per shoot. The yield component concept is difficult to apply to seed-established swards of alfalfa because the number of plants per area is unknown unless roots and crowns are excavated, which results in destruction of the plot (Volenec et al., 1987). That's why transplanting of individual plants into the field has been an effective method for establishing alfalfa at specific plant populations.

Several studies on alfalfa seeding rates indicated that seeding rates above 11.2 kg ha^{-1} did not significantly increase yield (Williams, 1917; Willard et al., 1934; Carmer and Jackobs, 1963; Zaleski, 1959). Alfalfa research conducted in Ohio by Williams (1917) indicated nonsignificant increases in forage production from rates of seeding higher than 11.2 kg ha^{-1}. Other research conducted in Ohio by Willard et al. (1934) with seeding rates of 2.8 to 56 kg ha^{-1} indicated no significant increase in forage production for rates greater than 11.2 kg ha^{-1}.

The plant density required for maximum yield in the first harvest year varies depending on the area and climate, 140 plants m^{-2} in California (Marble and Peterson, 1981), 140 plants m^{-2} in Michigan (Tesar, 1977; 1978), 230 plants m^{-2} in Ohio (Van Keuren, 1973), and 260 plants m^{-2}in Illinois (Jackobs and Miller, 1970).

According to Tesar and Marble (1988) approximately 150 to 250 plants m^{-2} are necessary for maximum yields in the year after seeding and 40 to 60 plants m^{-2} may be adequate for maximum yields in older stands provided the stands are uniform. More recently, data from South Dakota (Twidwell and Kephart, 1991) suggest that 110-165 plants m^{-2} are adequate for maximum yield.

Previous studies have shown that as seeding rates increase the initial number of seedlings established increases, but plant survival decreases, often resulting in ultimately equal plant densities for low and high seeding rates (Carmer and Jackobs, 1963; Meyer, 1978; Sund and Barrington, 1976; Van Keuren, 1973). Van Keuren (1973) reported a marked increase in plant numbers from high seeding rates compared with lower rates during the seeding year, but by spring of the following year the 13.5, 20.2, 27, 40.4, and 53.9 kg ha^{-1} had similar plant counts in North Dakota, USA. According to Bolger and Meyer (1983), percent plant mortality increased with plant density and losses were marked even in the low densities (i.e., 11 to 484 plants per m^{-2}). Fribourg and Kennedy (1953) found a sharp reduction in alfalfa plant numbers during the first winter and a continued reduction in stand density through the second harvest year to a final count of 11 to 56 plants m^{-2} in the USA.

Carbohydrates are the primary source of reserve energy stored in the vegetative organs of biennial and perennial forage plants. Stored food reserves in the roots and crown of alfalfa are an essential requirement for regrowth of shoots in the spring, initial regrowth after harvest, and for maintenance of crowns and roots during the winter (Smith, 1972). According to Ouellet (1977) in Canada, possibly the two most important factors causing winter injury are low air temperature and lack of snow cover, both of which influence soil temperature, and alfalfa stand loss is largely due to lethal soil temperature.

Work by Sund and Barrington (1976) found that the root % TNC among various rates of seeding (i.e., 6.7 - 40.4 kg ha^{-1}) in each of the sample years were quite similar, however, the amount of TNC available for survival over winter was substantially higher in the roots from the 6.7 kg ha^{-1} of seeding rate because of their larger size

and declined progressively by rate to the lowest amount in the 40.4 kg ha^{-1} seeding rate having the smallest roots. In study in P.E.I., Suzuki (1991) reported that the concentration of TNC in the crown and roots decreased slighly with age.

As a cold hardiness test, LT 50 is expressed as the lethal temperature at which 50 % of the population is killed. This method is a relative measure of the degree of temperature stress which the plants can tolerate. According to Suzuki (1991) young stands (2-4 yr of stand age) were slightly more cold hardy than the mid-age stands (5-7 yr of stand age) in 1987, but the difference was insignificant in 1986 and no difference in LT 50 was found between mid-age and old stands (8-10 yr of stand age).

Shoot morphology can be influenced by plant spacing. Recent studies in Indiana (Volenec et al., 1987) found that different population densities (i.e., 11, 22, 43, 97, and 172 plants m^{-2}) influenced shoot morphology. Averaged across cultivars (BIC, HI-PHY, and Vernal) yield per shoot, shoot number per plant, nodes per stem and stem diameter decreased as plant population increased. They also found that as plant population densities increased, the direct effect of yield per shoot (YPS) became a more important determinant of yield per plant when compared to shoots per plant. Mowat (1967) also reported that stems were thinner at a high density.

Alfalfa stem diameter and its branching was decreased in alfalfa stands established using high seeding rates (Hansen and Krueger, 1973; Krueger and Hansen, 1974; Radei, 1974). Sund and Barrington (1976) also showed that a more dense stand had fewer stems per plant, with near equal numbers of stems per unit area for the different seeding rates (i.e., 6.7, 13.4, 20.2, 26.9, or 40.4 kg ha^{-1}).

The influence of plant population on forage quality is not well understood. Bolger and Meyer (1983), using stands seeded in 1980, reported no effect of plant population (i.e., 11, 22, 33, 44, 55, or 100 plants m^{-2}) on concentrations of crude protein or ADF of alfalfa sampled in 1981. Meyer (1985) indicated that concentrations of ADF, acid detergent lignin (ADL), and NDF were unaffected by plant population even though shoot production ranged from 19 to 33

shoots per plant. According to McGuire (1981) the crude protein content was not influenced by seeding rates of 8.4, 11.2, 16.9, or 22.5 kg ha^{-1} or row width of 6.7 or 13.4 cm in cultivars Vernal and DuPuits during 1963-1964 in Ohio, USA. Buxton and Hornstein (1986) reported that a negative association between IVDMD and lignin contrations of both alfalfa stems and herbage. Sund and Barrington (1976) also reported that seeding rates of 6.7 to 40.4 kg ha^{-1} did not influence cell wall constituents (CWC), ADF, or ADL in Wisconsin, USA. Van Keuren (1973) found that no effect of seeding rates (i.e., 3.4 - 54 kg ha^{-1}) on lignin, dry matter digestibility, or cellulose content in Ohio, USA.

The objectives of this study were: 1) to determine the optimum alfalfa population density having high yield, quality and persistence for the dehydrated alfalfa industry; 2) to compare the gross morphology, forage quality, and winter survival of cultivars Algonquin and Vernal when grown at a range of stand densities; 3) to investigate the interrelationships among stand density, individual plant size, carbohydrate storage, and the ability of alfalfa to overwinter.

3.2 Materials and Methods.

Two sets of test plots were established in each seeding year. One set of plots (using transplanted seedings) was established to follow changes in yield and morphological characteristics over a number of years. The second set of plots (direct seeded) were used for destructive harvesting to measure population effects on carbohydrate storage and the ability of the plants to winterharden.

3.2.1. Transplant plot.

3.2.1.1. Plant material preparation in the greenhouse.

The root trainers (1 x 1 x 4 inch, ' Fives ', Spencer- Lemaire Industries Ltd.) were filled with a soil mixture composed of peat moss, black soil, and coarse sand (1 : 1 : 1). In order to monitor cultivar differences, approximately 8,800 seeds of a relatively old cultivar, Vernal, and of a recently released cultivar, Algonquin were pre-inoculated with *Rhizobium meliloti* and planted into root trainers in 1992 and 1993. The plants were allowed to germinate and grow for five weeks in the greenhouse at 20 ° C and a 16 h photoperiod. Plants were watered daily and fertilized biweekly with a liquid fertilizer (N : P_2O_5 : K_2O, 20 : 20 : 20). The fungicide No-Damp was sprayed twice, i.e., on one day after planting and after emergence, in order to control damping-off.

3.2.1.2. Transplanting in the field.

After five weeks growth in the greenhouse, seedlings were transplanted equidistantly into 2.25 m^2 (1.5 x 1.5 m) plots at distances of 6, 10, 15, and 25 cm from each other. These spacings represent population densities of 278, 100, 45, and 16 plants per m^2. Transplanting occurred on June 15, 1992 and May 31, 1993. Due to

the difficulty of transplanting seedlings at the close distance of 4.5 cm corresponding to 494 plants m-2, plots were seeded by hand into a grid using previously prepared templates. The experimental design was a split plot design; the main plots were composed of the cultivars Algonquin and Vernal, and the sub-plots were composed of five different spacings (i.e., 4.5, 6, 10, 15, and 25 cm).

3.2.1.3. Harvests for yield.

In the seeding year, transplant plots which were established in 1992, were harvested once at approximately the late seed pod stage on September 15, 1992 and at the full bloom stage on August 19, 1993.

In the first production year of alfalfa, which was transplanted in 1992, plants were harvested at one tenth bloom stage on June 18, 1993 at the first harvest and at one tenth bloom stage on August 18, 1993 at the second harvest. To estimate yield per ha 65, 40, 15, 7, and 3 plants per plot were clipped from the center area of 4.5, 6, 10, 15, and 25 cm plant spacing plots, respectively, in each cultivar to a stubble height of 5 cm. Harvested plant material was dried at 65C for 3 days in a forced air dryer and then weighed.

3.2.1.4. Harvests for morphological characteristics.

At each harvest 15, 13, 6, 3, and 2 plants from the plant spacings of 4.5, 6, 10, 15, and 25 cm, respectively, were clipped by hand using scissors to a stubble height of 5 cm. From these clipped plants, shoots per plant were counted, and 15 shoots were randomly selected and yield per shoot was calculated by dividing the total dry weight of fifteen shoots (leaves plus stems) by fifteen.

Of the 15 shoots selected above, stem diameter at the lowest node, stem length, node number per shoot, leaf area per plant, and leaf to stem ratio were measured. In order to measure leaf area leaves were separated from stems and measured with a LI 3100

leaf area meter (Li-Cor Ltd, Lincoln, Nebraska). Leaf to stem ratio was calculated after drying the leaves and stems separately at 65C for 3 days.

3.2.1.5. Winter survival.

The number of plants per plot in each cultivar within a 50 cm by 2 rows randomly selected area were counted in mid September, 1992 and again in late April, 1993 to assess winter survival. The percent winter survival was calculated as :

% winter survival = (plant numbers in the spring / plant numbers in the fall) x 100.

3.2.1.6. Quality analysis.

The shoot samples were ground with a cyclone mill through 1 mm mesh screen. Samples from the fall harvest during the establishment year of 1992 and the first and second harvest in the first production year of 1993 were analyzed for crude protein, acid detergent fiber (ADF) and neutral detergent fiber (NDF) by Norwest Labs Edmonton, Canada. Crude protein was analyzed by a Leco Nitrogen Analyzer (LECO FP 428, St. Joceph, MI, USA). The method used for determining protein is an official AOAC method (AOAC, 1990). Acid detergent fiber was analyzed by the AOAC method (AOAC, 1990). Neutral detergent fiber was analyzed by the method of Goering and Van Soest (1970).

3.2.2. Seeded plot.

3.2.2.1. Seeding in the field.

To determine the cold hardiness of Algonquin and Vernal at a range of plant spacings, plots were seeded by hand on June 1, 1992

using previously prepared grid templates. Plot sizes varied in order to provide enough plant material for TNC, TEG, and LT 50 estimates. The plot sizes were 2.25 (1.5 x 1.5 m), 2.25 (1.5 x 1.5 m), 4.0 (2.0 x 2.0), 9.0 (3.0 x 3.0), and 16.0 (4.0 x 4.0 m) m^2 for 4.5, 6.0, 10, 15, and 25 cm plant spacing. The cultivars Algonquin and Vernal were seeded and the plants were thinned to one plant per 4.5, 6, 10, 15, and 25 cm, after 6 weeks growth. Reseeding was done where necessary to ensure a completed grid of plants. Plants were irrigated after seeding to facilitate establishment.

3.2.2.2. Total Nonstructural Carbohydrates.

Five plants were dug from each plot on October 4, 1992 prior to freeze up and on April 16, 1993. After digging the shoots were clipped to 5 cm and the roots were clipped to 8 cm. The plant material was washed free of soil and any dead material was trimmed away. The plant material was then oven-dried at 75C and ground to pass through a 1 mm screen.

200 mg of ground alfalfa roots with crowns, and 50 ml of 0.2 N H_2SO_4 were added to a 125 ml flask and heated at 100 C for one hour. The digested solution was then filtered through Whattmann #40 filter paper and the residue was washed with hot distilled water. The filtrate and washings were combined, cooled, and diluted to a volume of 100 ml with distilled water (Suzuki, 1971). 1 ml of extract in a small Erlenmyer flask was mixed with 12 ml distilled water. 2 ml were removed into a small test tube and 1 ml of 5 % phenol and 5 ml of concentrated sulfuric acid were added to the test tube in a fume hood. The test tubes were left for 5 minutes in the fume hood and then cooled in a water bath for 15 minutes. Reducing sugars were then determined by reading at a wave length of 490 nm on a spectro-photometer. Glucose was used as a standard (Dubois et al., 1956). Carbohydrate content was expressed as a percent of carbohydrates on a dry matter basis.

3.2.2.3. Total Etiolated Growth.

On October 4, 1992 prior to freeze up and on April 16, 1993, five alfalfa plant crowns and roots of Algonquin and Vernal were excavated from each seeded plot established in 1992. Shoots were clipped to 5 cm and roots with crown were clipped to 8 cm. The plant material was washed free of soil and any dead plant material was removed. The plant material was blotted dry and weighed. The plants were potted in 10 cm diameter by 25 cm deep plastic pots, filled with moist vermiculite, and placed in a growth room without light, at 20C, and a relative humidity of 80 %. The etiolated shoots were harvested at 2-week intervals until no further growth occurred (Mckenzie et al., 1988). Shoots were oven-dried at 70C for 48 h and weighed. TEG is an indirect measure of carbohydrate reserves and is expressed as milligrams dry weight of etiolated growth per initial fresh weight of crown-root tissue.

3.2.2.4. LT 50.

Twenty five plants of Algonquin and Vernal from each plot were dug up on October 4, 1992, prior to freeze up and placed into cloth bags. The soil was removed from the roots, and the shoots were clipped to 5 cm where the roots were clipped to 8 cm. The plants were potted in 10 cm diameter by 25 cm deep plastic pots filled with vermiculite. Five plants from the 25 plants were selected and cooled to -4, -8, -12, -16, or -20 C for the freezing test. The plants were cooled at a rate of 3-4 C per hour. After the plants attained the desired test temperatures measured by placing thermo-couples into the upper vermiculite, the pots were removed from the freezing cabinet and placed into a 5C refrigerator overnight to facilitate slow rewarming. Plants in the pots were scored for regrowth ten days later (McKenzie and McLean, 1980). The LT 50 temperature was defined as that temperature at which 50 % of plants were killed.

3.2.3. Statistical analysis.

Data analysis was carried out by ANOVA procedures using GLM procedures of SAS (SAS Institute Inc., 1989). The plant spacing effects were partitioned into linear and quadratic components using orthogonal polynominal coefficients. The ANOVA tables can be found in the appendix.

3.2.4. Weather conditions.

The average monthly temperatures ((maximum + minimum) / 2) were recorded at the Edmonton International Airport by Environment Canada in Alberta from June, 1992 to August, 1993 (Appendix Fig. a.1). Generally, the highest air temperature occurred between mid-July and early-August. However, the air temperature during this period of 1992 to 1993 was a little lower than in 1991 or the 30 year average.

The monthly minimum temperatures during the period of early-December, 1992 to late-January, 1993 was on average colder than the winter of 1991-1992, and colder than the 30 year average The lowest monthly minimum temperature occurred in December, 1992 and January, 1993 (Appendix Fig. a.2).

The monthly maximum temperatures during the period of December, 1992 to April, 1993 were lowerer than the winter of 1991 to 1992 (Appendix Fig. a.3).

In terms of the total monthly precipitation during June, 1992, the time of establishment, it was much drier than the establishment year of 1993 and it was also much drier than the 30 year average. Generally it rained more in 1993 during the growing season than 1992 (Appendix Fig. a.4).

3.3 Results.

3.3.1 Yield.

The yield of alfalfa harvested on September 15 during the establishment year of 1992 is given in Fig.1. There was a significant ($p<0.05$) quadratic effect of plant spacing on yield for each cultivar. There was no significant effect of cultivar and no significant cultivar x plant spacing interaction. The least yield was attained at 4.5 cm plant spacing in each cultivar. This may be due a different rate of establishment. The plants grown at a 4.5 cm plant spacing were seeded one week after the other seedlings grown at 6, 10, 15 and 25 cm plant spacings, were transplanted. For alfalfa plants, transplanted in 1993, there was a significant ($p<0.01$) linear and ($p<0.05$) quadratic effect of plant spacing on yield in Algonquin and Vernal (Fig.3.2). The greatest yield was attained at the 10 cm plant spacing in Algonquin and a 4.5 cm plant spacing in Vernal, and the lowest yield was attained at the 25 cm plant spacing in both cultivars in the fall harvest during the establishment year. There was no significant effect of cultivar and cultivar x plant spacing interaction on yield.

In the first production year of 1993 there was a significant ($p<0.01$) cultivar effect on yield. Vernal had a higher yield than Algonquin at the first harvest. There was also a significant ($p<0.05$) linear and ($p<0.01$) quadratic relationship between plant spacing and yield (Fig.3.3). However, there was no significant cultivar x plant spacing interaction. The greatest yield was attained at plant spacing of 15 cm and the lowest yield at the 25 cm plant spacing in Algonquin. In Vernal the greatest yield was from plants grown at the 10 cm plant spacing and the lowest yield at the 25 cm plant spacing.

In the second harvest, there was no significant cultivar effect and no cultivar x plant spacing interaction. There was also no effect of plant spacing on yield (Fig.3.4).

For annual total yield there was a significant ($p<0.01$) cultivar effect. Vernal showed a little higher yield than Algonquin (Fig.5). Although there was a significant ($p<0.05$) linear relationship between plant spacing and yield at the first harvest, there was no significant

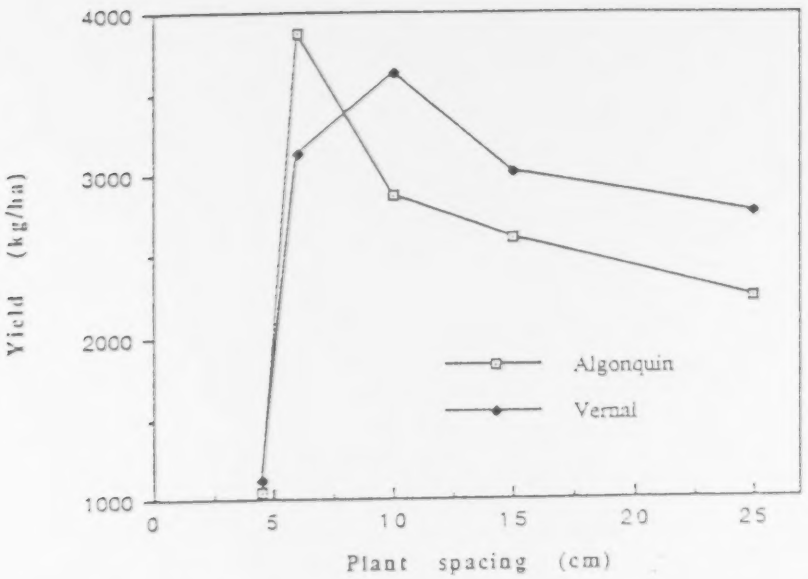


Figure 3.1. Effect of plant spacing on yield in Algonquin and Vernal in the fall of establishment year of 1992.

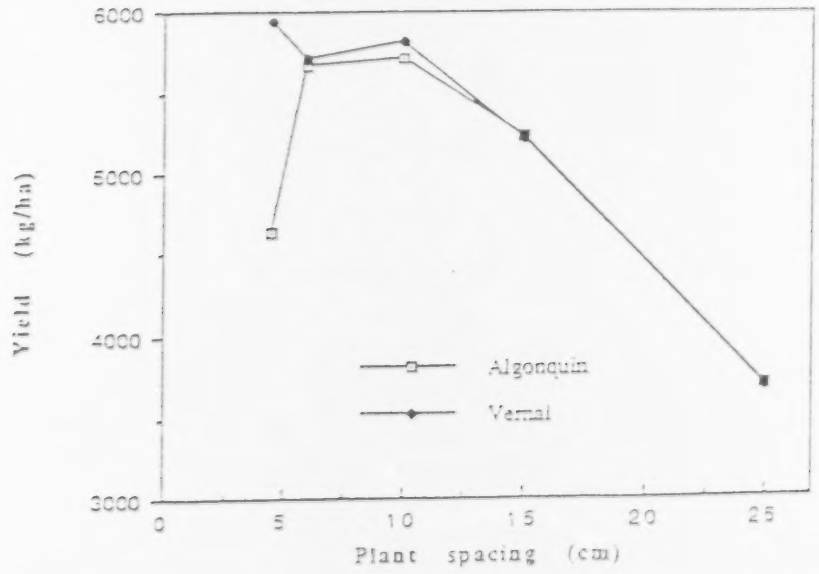


Figure 3.2. Effect of plant spacing on yield in Algonquin and Vernal in the fall of establishment year of 1993.

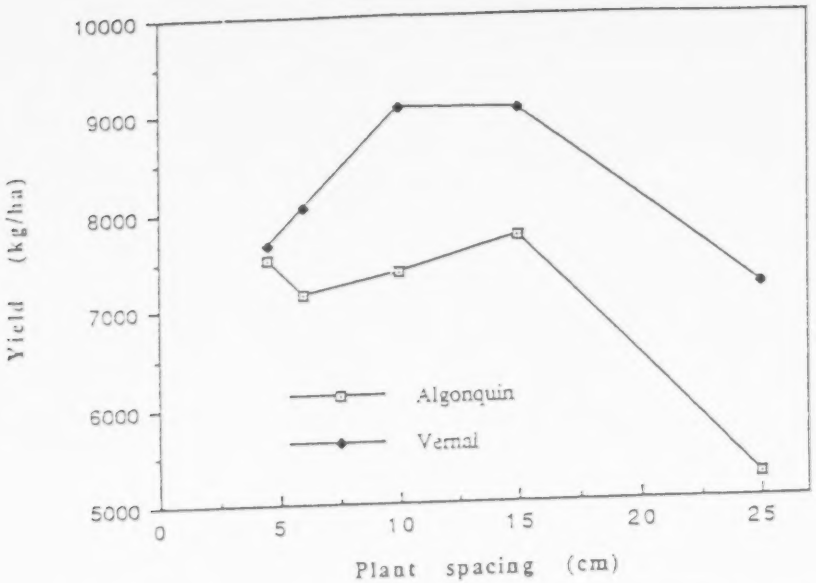


Figure 3.3. Effect of plant spacing on yield in Algonquin and Vernal at the first harvest in the first production year of 1993.

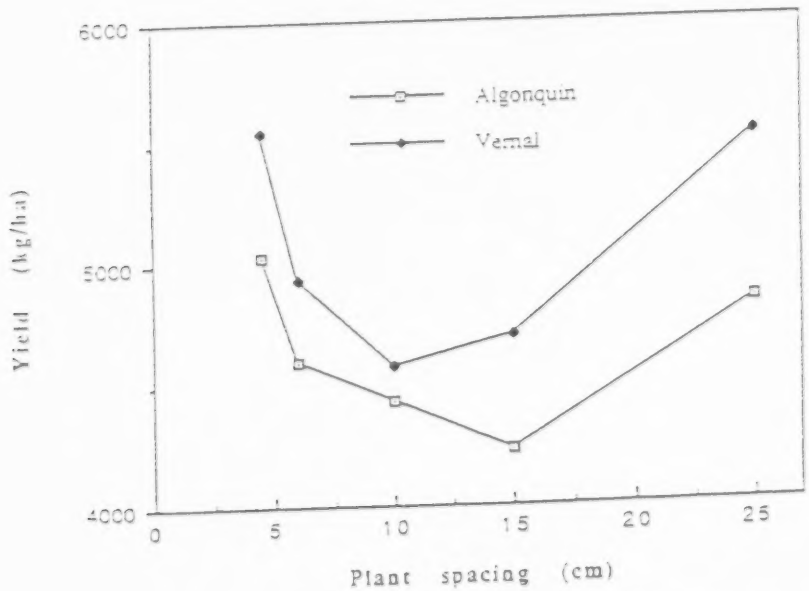


Figure 3.4. Effect of plant spacing on yield in Algonquin and Vernal at the second harvest in the first production year of 1993.

effect of plant spacing on yield in regards to the annual total yield.

3.3.2. Winter survival

Winter survival was over 93 % at all plant spacings (Fig.3.6). Representative samples of 4.5, 15, and 25 cm plant spacings are illustrated in Fig.3.7. Vacant spaces are not visible at the 4.5 cm plant spacing; this reveals the high level of winter survival. There was a significant ($p<0.01$) linear effect of plant spacing on winter survival. Winter survival increased with increasing plant spacing. There were no dead plants at 15, 20, or 25 cm plant spacing in either cultivar. There was no significant cultivar effect and no significant cultivar x plant spacing interaction.

3.3.3. Cold hardiness.

3.3.3.1. Total Nonstructural Carbohydrates (TNC)

There was a significant ($p<0.05$) quadratic effect of plant spacing on % TNC in both cultivars in early October, 1992 (Fig.3.8). Variation was from 18.9 to 22.7 %. There was no significant effect of cultivar or cultivar x plant spacing interaction on % TNC. However, when % TNC was multiplied by dry root weight, grams TNC showed a significant ($P<0.01$) linear effect of plant spacing (Fig.3.9). Thus, the amount of TNC available for survival over winter was substantially higher in the roots of the plants grown at the 25 cm plant spacing, and declined progressively with the lowest amount in the 4.5 cm plant spacing. The greatest increase in grams of TNC occurred between the 15 and 25 cm plant spacing. But there was no significant effect of cultivar, or cultivar x plant spacing interaction.

There was no significant difference between cultivars and plant spacings for % TNC in the spring of 1993 (Fig.3.10). However, there was a significant ($p<0.001$) linear relationship between plant spacing and grams of TNC per root (Fig.3.11). As plant spacing increased, the

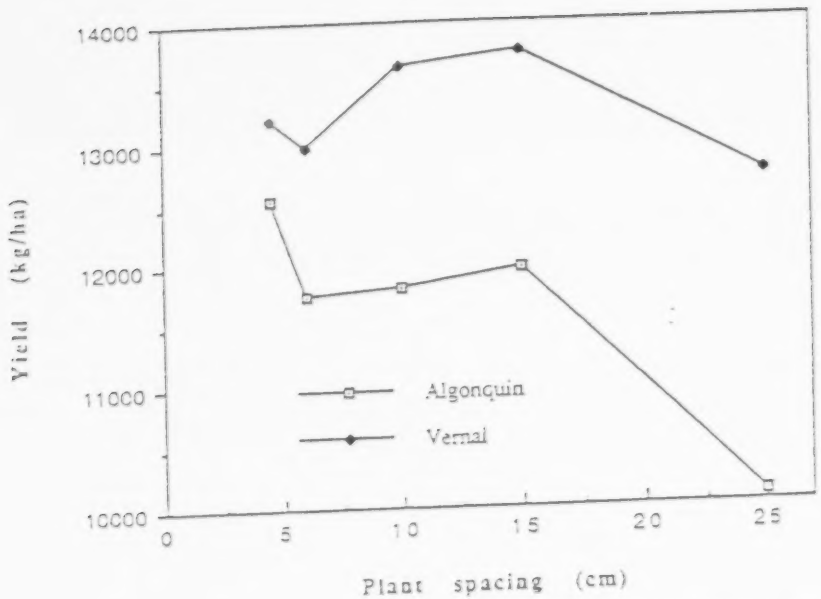


Figure 3.5. Effect of plant spacing on total annual yield in Algonquin and Vernal in the first production year of 1993.

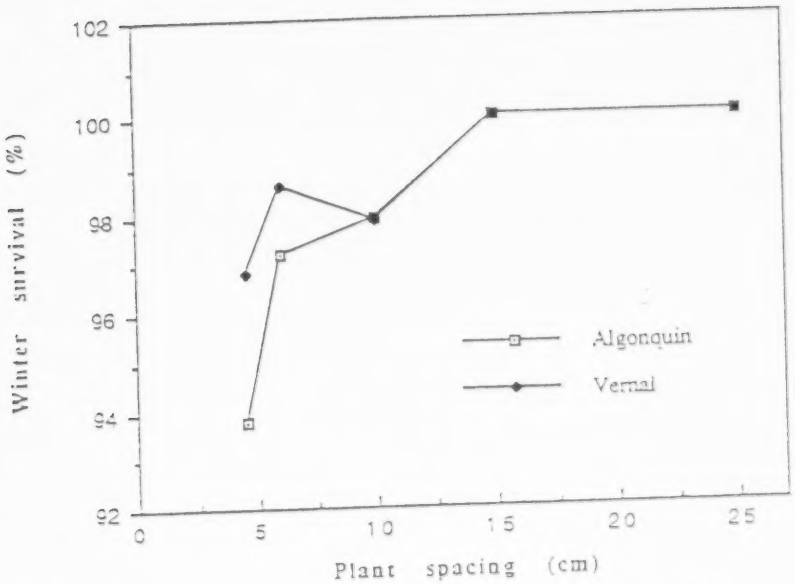
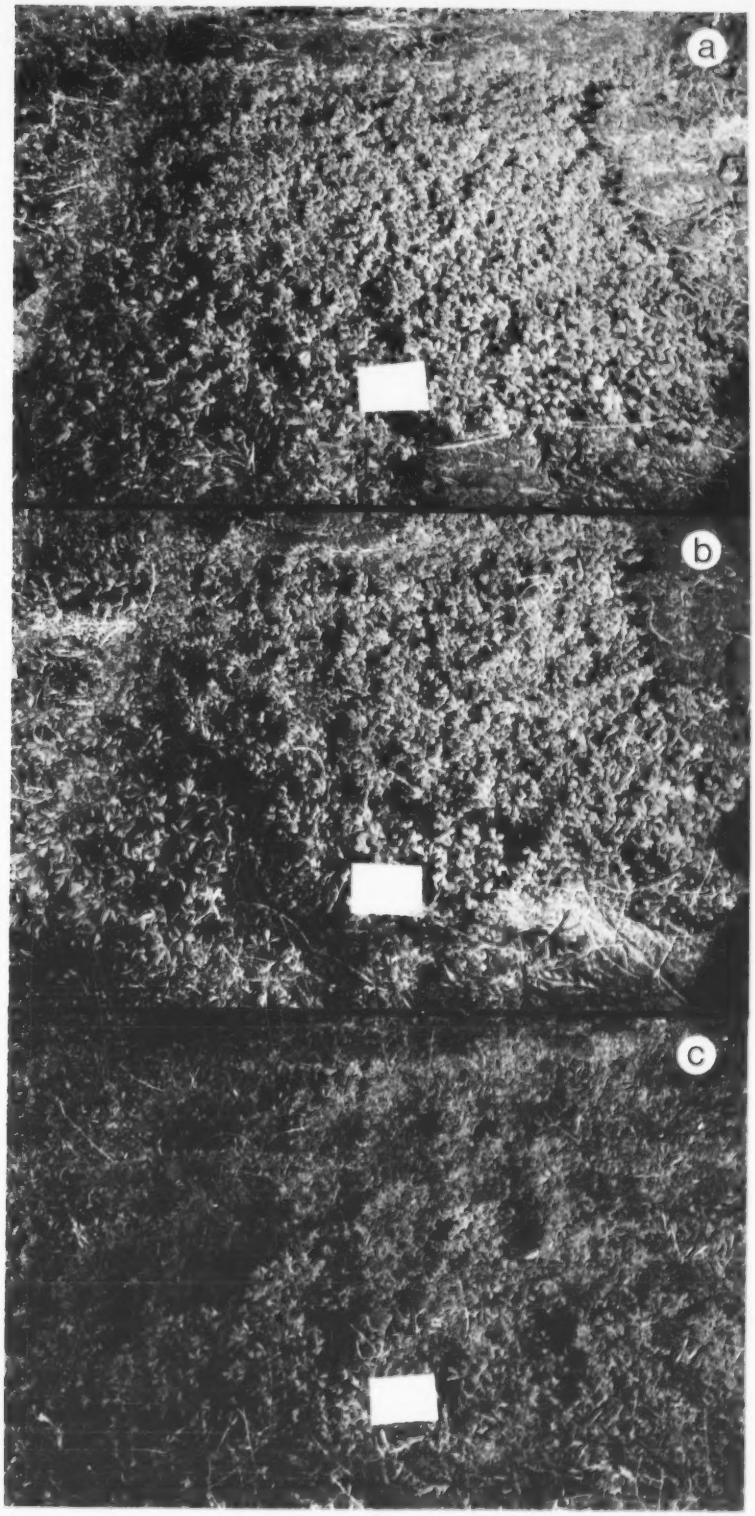


Figure 3.6. Effect of plant spacing on winter survival in Algonquin and Vernal in the spring of 1993 after first winter.

Figure 3.7. Representative samples of alfalfa plants as seen in the fall of 1993 under different plant spacings : a) 4.5 cm, b) 15 cm, and c) 25 cm, respectively.


a
b
c

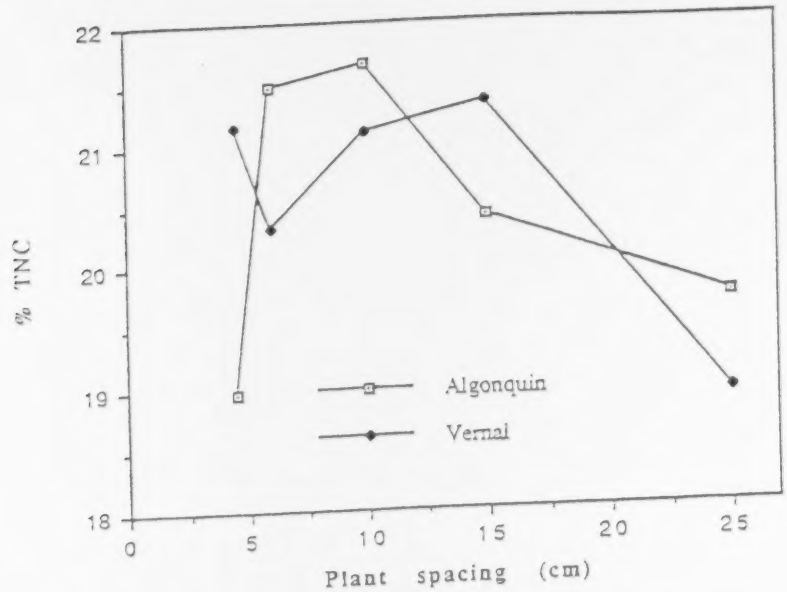


Figure 3.8. Effect of plant spacing on % TNC in Algonquin and Vernal in the fall of establishment year of 1992.

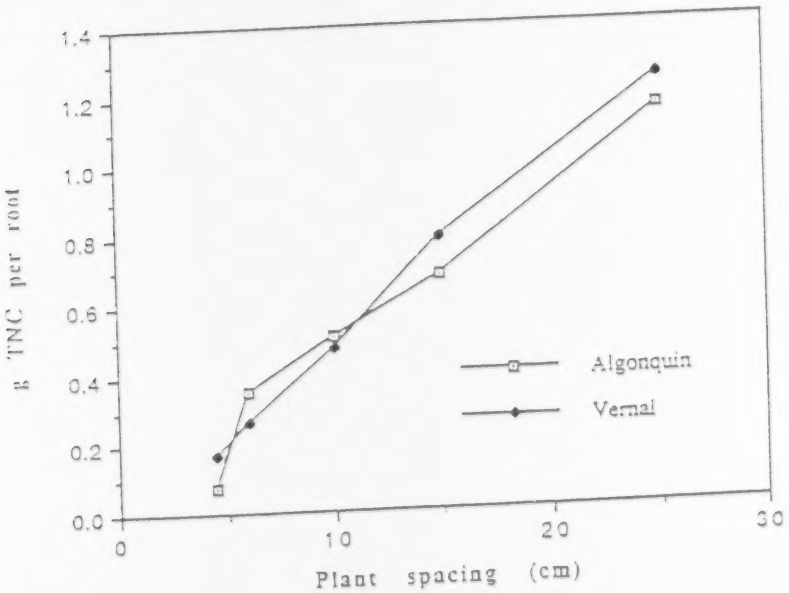


Figure 3.9. Effect of plant spacing on grams TNC per root in Algonquin and Vernal in the fall of establishment year of 1992.

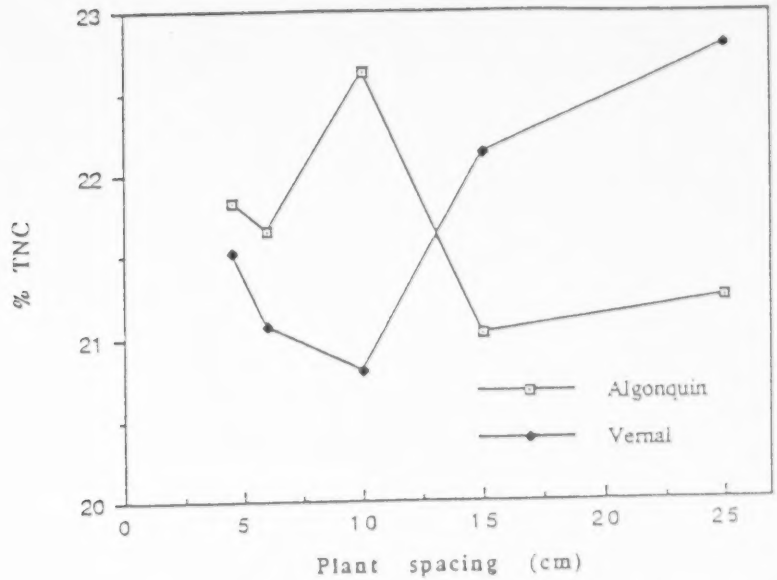


Figure 3.10. Effect of plant spacing on % TNC in Algonquin and Vernal in the spring of 1993 after first winter.

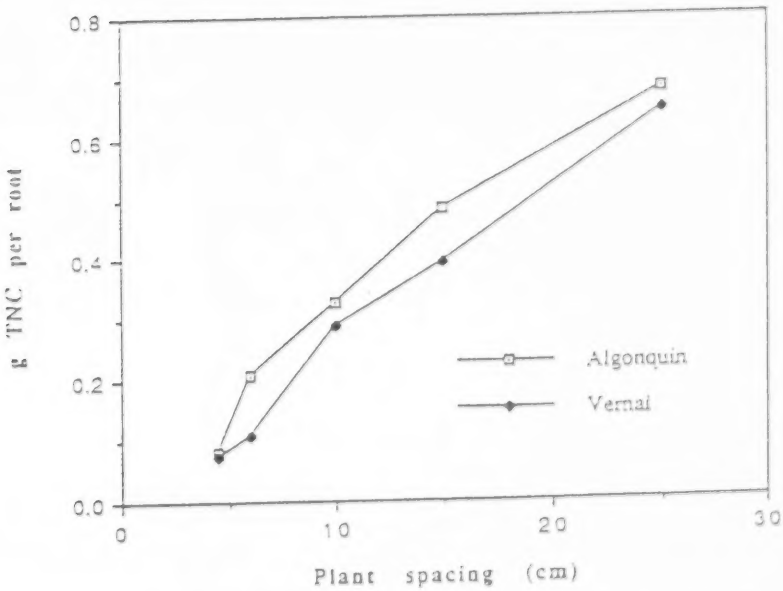


Figure 3.11. Effect of plant spacing on grams TNC per root in Algonquin and Vernal in the spring of 1993 after first winter.

grams of TNC per root increased. The TNC per root was higher at the 25 cm plant spacing, and least at the 4.5 cm plant spacing in both cultivars. There was no significant effect of cultivar or cultivar x plant spacing interaction on grams of TNC per root.

3.3.3.2. Total Etiolated Growth (TEG)

There was a significant ($p<0.001$) linear and ($p<0.001$) quadratic effect of plant spacing on TEG in the fall of 1992. As plant spacing increased TEG generally decreased (Fig.3.12). The highest TEG was attained at the 4.5 or 6 cm plant spacing in the cultivars Vernal and Algonquin, respectively, and the least TEG occurred at 25 cm plant spacing in both cultivars. There was no significant differences between cultivars and no cultivar x plant spacing interaction on TEG.

In the spring of 1993, TEG showed no significant response to plant spacing, cultivar, or cultivar x plant spacing interaction (Fig.3.13). In both cultivars the greatest TEG was attained at a 4.5 cm plant spacing, but the least TEG was attained at a 6 cm plant spacing in Algonquin and at a 15 cm plant spacing in Vernal. There was very little difference between the 10 and 25 cm plant spacing in each cultivar. The TEG measured in the fall of 1992 was much higher than that recorded in the spring of 1993 (Fig.3.12-3.13).

3.3.3.3. LT 50

There was a significant ($p<0.01$) linear relationship between plant spacing and LT 50. The lowest LT 50, -10.5 C occurred at the 25 cm plant spacing for both cultivars. The highest LT 50 was -6.5 C for Algonquin and -8.5 C for Vernal (Fig.3.14). Vernal was somewhat more cold hardy than Algonquin between the 4.5 and 6 cm plant spacing. However, there was no significant difference in LT 50 between cultivars or cultivar x plant spacing interaction.

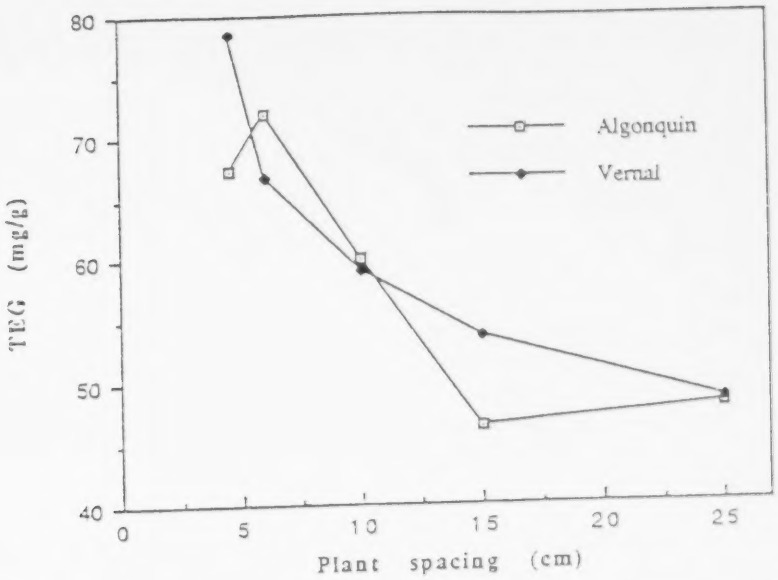


Figure 3.12. Effect of plant spacing on TEG in Algonquin and Vernal in the fall of establishment year of 1992.

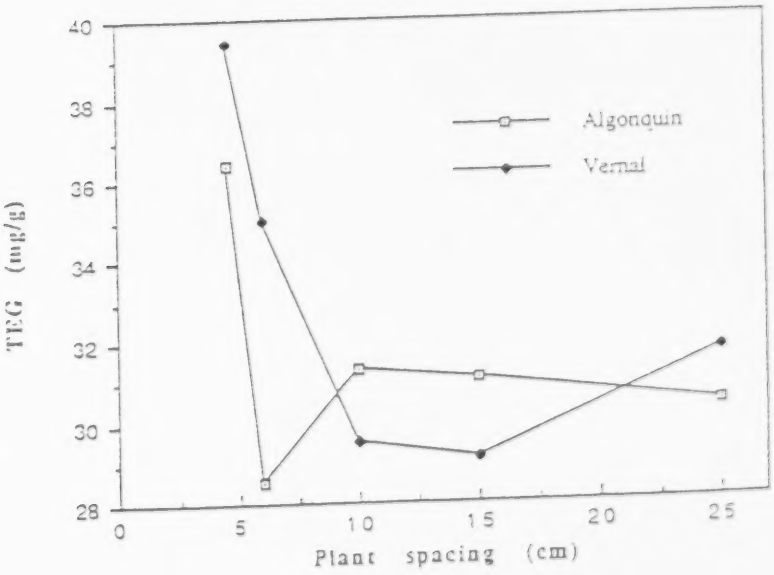


Figure 3.13. Effect of plant spacing on TEG in Algonquin and Vernal in the spring of 1993 after first winter.

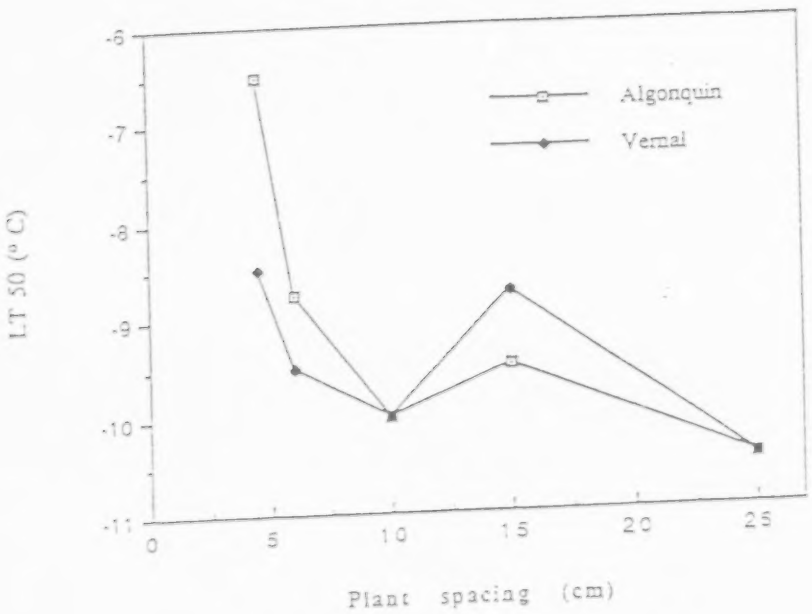


Figure 3.14. Effect of plant spacing on LT 50 in Algonquin and Vernal in the spring of 1993 after first winter.

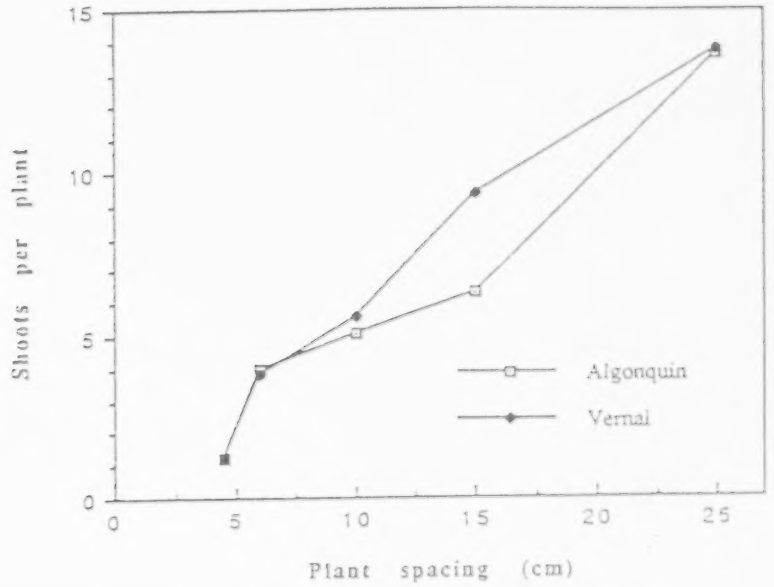


Figure 3.15. Effect of plant spacing on shoots per plant in Algonquin and Vernal in the fall of establishment year of 1992.

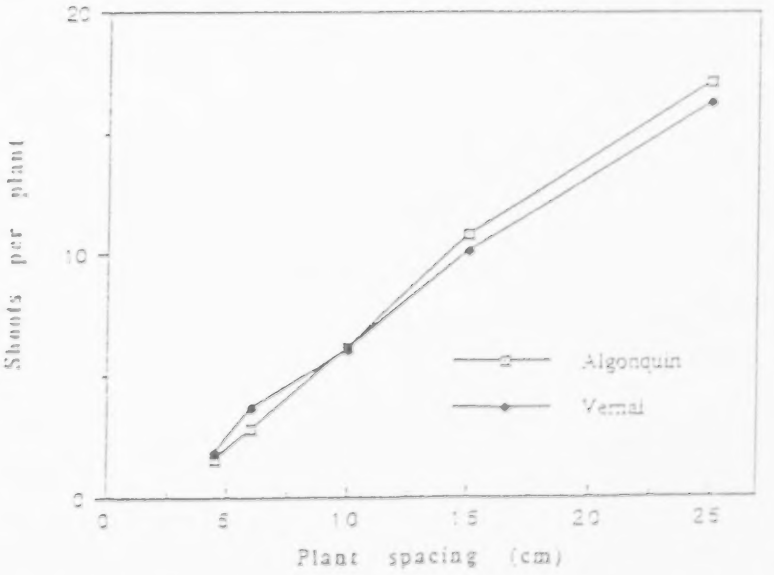


Figure 3.16. Effect of plant spacing on shoots per plant in Algonquin and Vernal in the fall of establishment year of 1993.

3.3.4. Morphology.

3.3.4.1. Shoots per plant.

There was a significant ($p<0.001$) linear effect of plant spacing on shoot number per plant in the fall harvest of 1992 the year of establishment (Fig.3.15). As plant spacing increased, shoots per plant increased. The highest number of shoots per plant occurred at the 25 cm plant spacing and the lowest number of shoots occurred at the 4.5 cm plant spacing in both cultivars. However, there was no significant effect of cultivar and no significant cultivar x plant spacing interaction. A similar response occurred in the 1993 establishment year, as plant spacing increased the shoots per plant increased linearly ($p<0.01$) (Fig.3.16). There was no cultivar effect or cultivar x plant spacing interaction on shoots per plant. After the first winter there was a significant ($p<0.001$) linear effect of plant spacing on shoots per plant in the spring of the first production year 1993.

Similar to the fall of 1992 there was no significant cultivar effect or cultivar x plant spacing interaction on number of shoots per plant in the first production year 1993. However, the number of shoots per plant in the spring of 1993 was higher than that in the fall of 1992 (Fig.3.17).

There was a significant ($p<0.001$) linear effect at the first harvest and significant ($p<0.001$) linear and ($p<0.001$) quadratic effect of plant spacing on shoots per plant at the second harvest in the first production year of 1993 (Fig.3.18 and 3.19). As plant spacing increased the shoots per plant increased at each harvest. As shown in Fig.3.18 and 3.19, the highest number of shoots per plant occurred at the 25 cm plant spacing and least at the 4.5 cm plant spacing at each harvest. The greatest increase in shoots per plant occurred between the 15 and 25 cm plant spacings at each harvest. The number of shoots per plant at the second harvest was a little higher than at the first harvest for each cultivar. There was no significant cultivar effect or cultivar x plant spacing interaction on number of shoots per plant at either harvest.

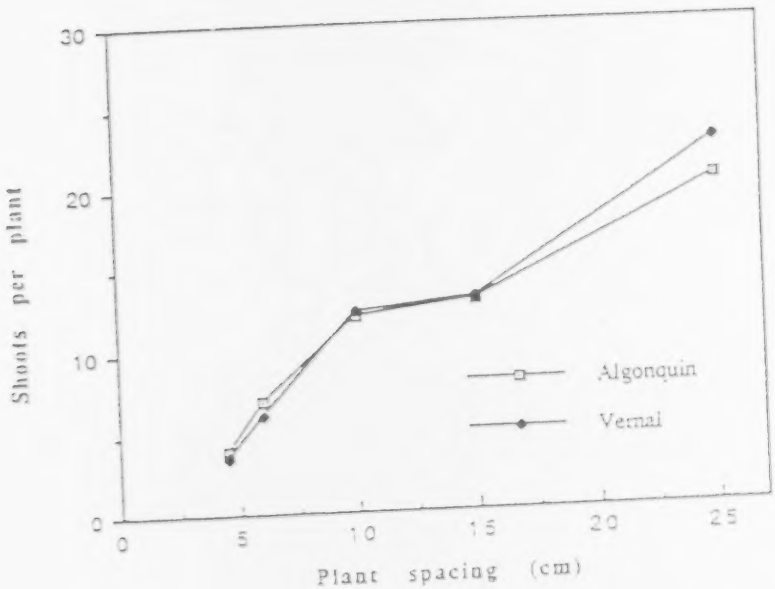


Figure 3.17. Effect of plant spacing on shoots per plant in Algonquin and Vernal in the spring of 1993 after the first winter.

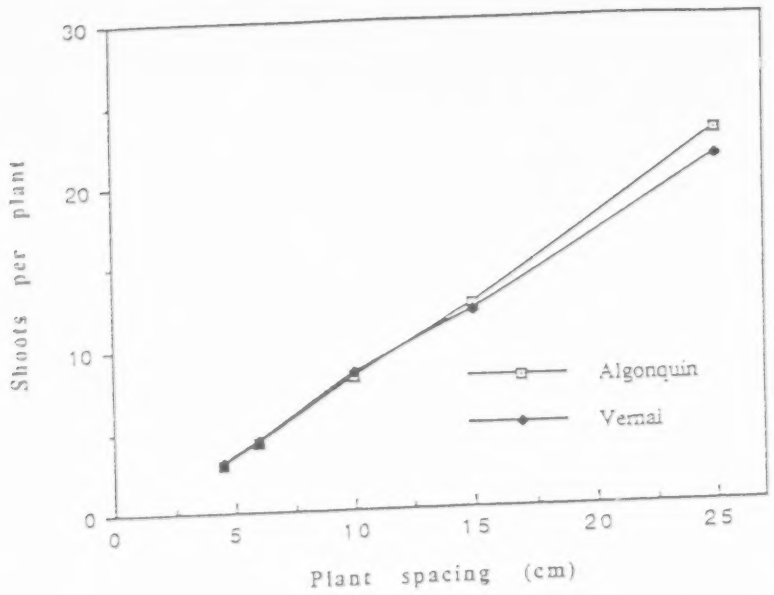


Figure 3.18. Effect of plant spacing on shoots per plant in Algonquin and Vernal at the first harvest in the first production year of 1993.

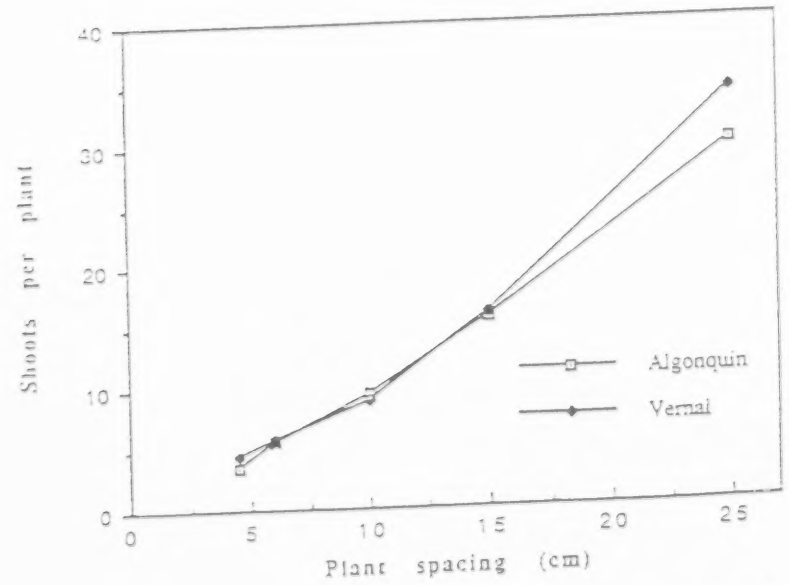


Figure 3.19. Effect of plant spacing on shoots per plant in Algonquin and Vernal at the second harvest in the first production year of 1993.

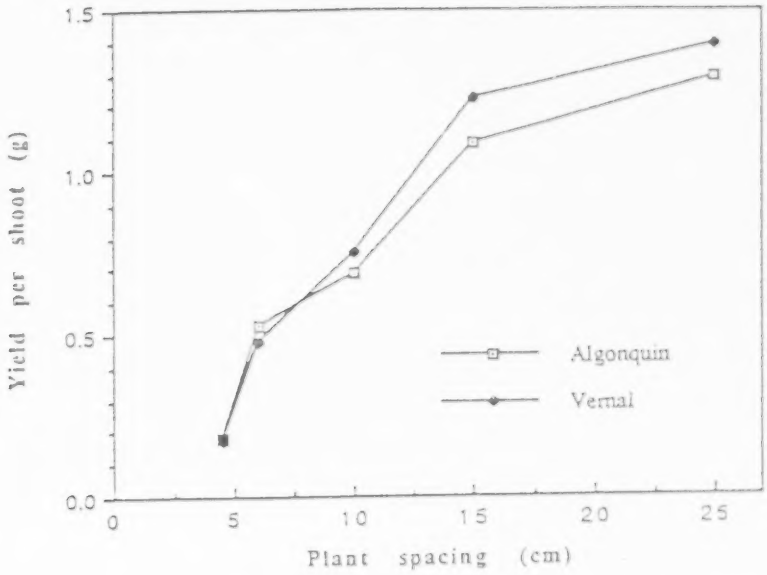


Figure 3.20. Effect of plant spacing on yield per shoot in Algonquin and Vernal in the fall of establishment year of 1992.

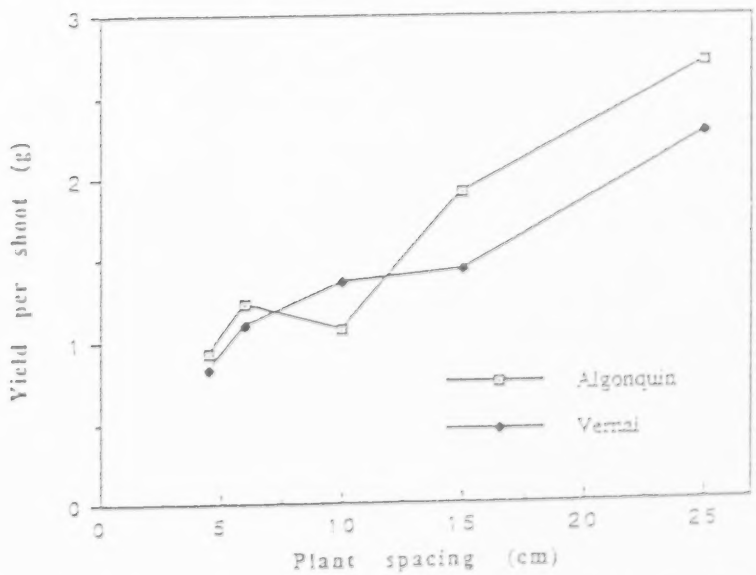


Figure 3.21. Effect of plant spacing on yield per shoot in Algonquin and Vernal in the fall of establishment year of 1993.

3.3.4.2. Yield per shoot

As plant spacing increased yield per shoot increased in each cultivar. There was a significant ($p<0.001$) linear and ($p<0.001$) quadratic effect of plant spacing on yield per shoot in the fall harvest of 1992 during the year of establishment (Fig.3.20). For alfalfa plants transplanted in 1993, there was a significant ($p<0.01$) linear effect of plant spacing on yield per shoot in both cultivars in the fall harvest during the establishment year. The largest yield per shoot was attained at the 25 cm plant spacing and the least at the 4.5 cm plant spacing in both cultivars (Fig.3.21). There was no significant effect of cultivar or cultivar x plant spacing interaction for yield per shoot.

A significant ($p<0.01$) linear effect of plant spacing on yield per shoot occurred at the first harvest, and a significant ($p<0.001$) linear and ($p<0.001$) quadratic effect of plant spacing on yield per shoot occurred at the second harvest in the first production year of 1993 (Fig.3.22-3.23). The greatest yield per shoot occurred when plants were grown at 25 cm plant spacing and least for plants grown at 4.5 cm plant spacing at each harvest. However, there was no significant effect of cultivar or cultivar x plant spacing interaction at any harvest during 1992 - 1993. After the first winter, with successive harvests, the yield per shoot at the second harvest was much lower than that of first harvest (Fig.3.22-3.23).

3.3.4.3. Stem diameter.

The average stem diameter measured in the fall harvests of the 1992 and 1993 establishment years, and in the first and second harvests in the first production year 1993 are given in Figs.3.24, 3.25, 3.26, and 3.27, respectively. Generally, as plant spacing increased the average stem diameter increased in each cultivar. There was a significant ($p<0.001$) linear and ($p<0.001$) quadratic effect of plant spacing on stem diameter in the fall harvest of 1992. In alfalfa plants transplanted in 1993, there was a significant

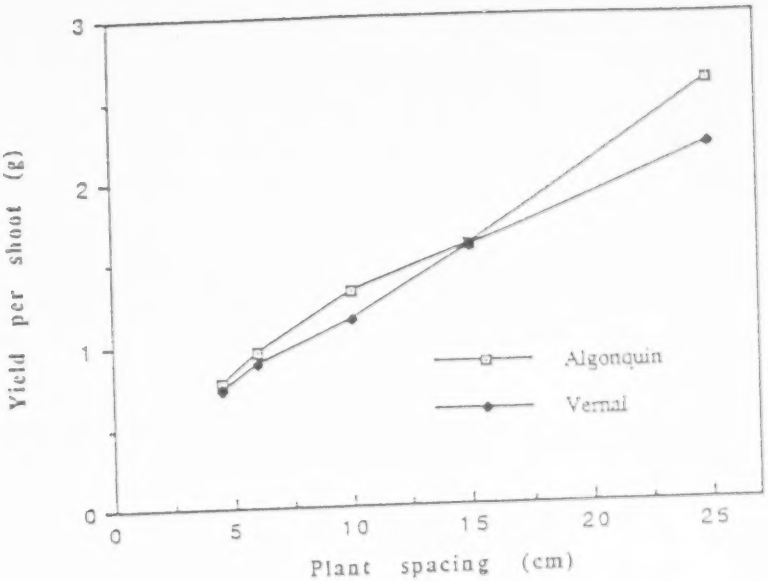


Figure 3.22. Effect of plant spacing on yield per shoot in Algonquin and Vernal at the first harvest in the first production year of 1993.

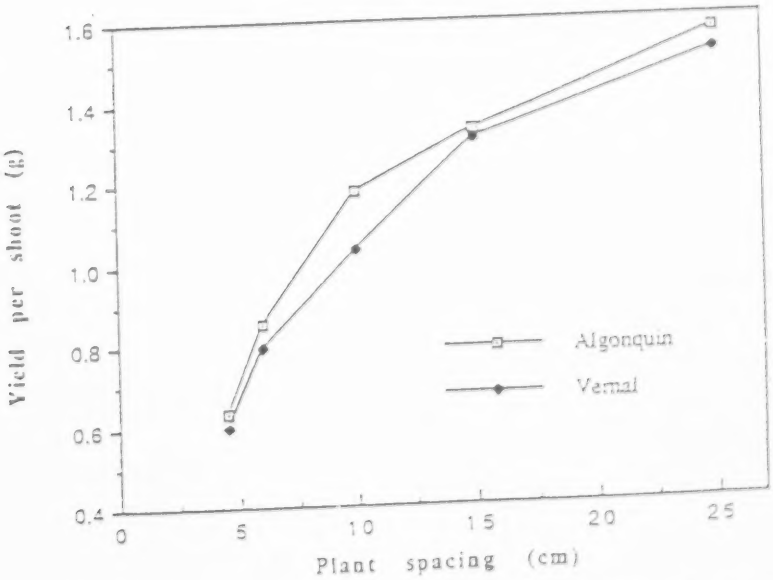


Figure 3.23. Effect of plant spacing on yield per shoot in Algonquin and Vernal at the second harvest in the first production year of 1993.

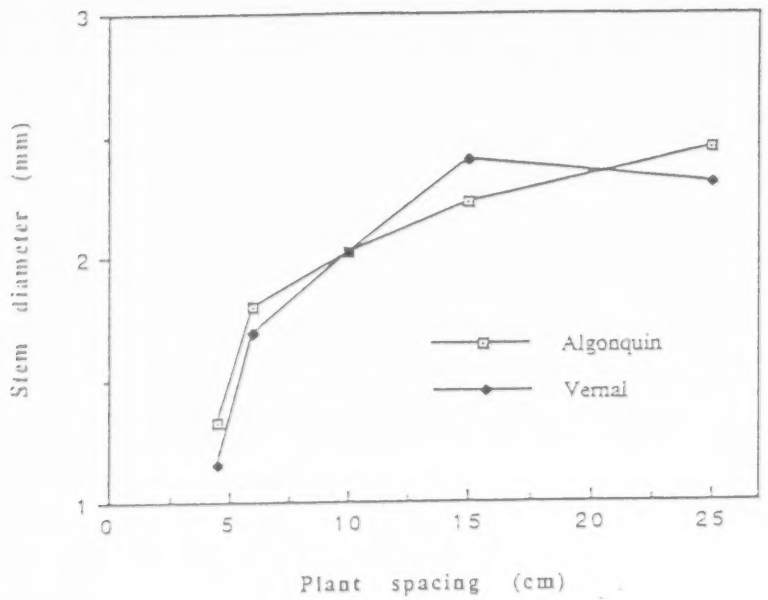


Figure 3.24. Effect of plant spacing on stem diameter in Algonquin and Vernal in the fall of establishment year of 1992.

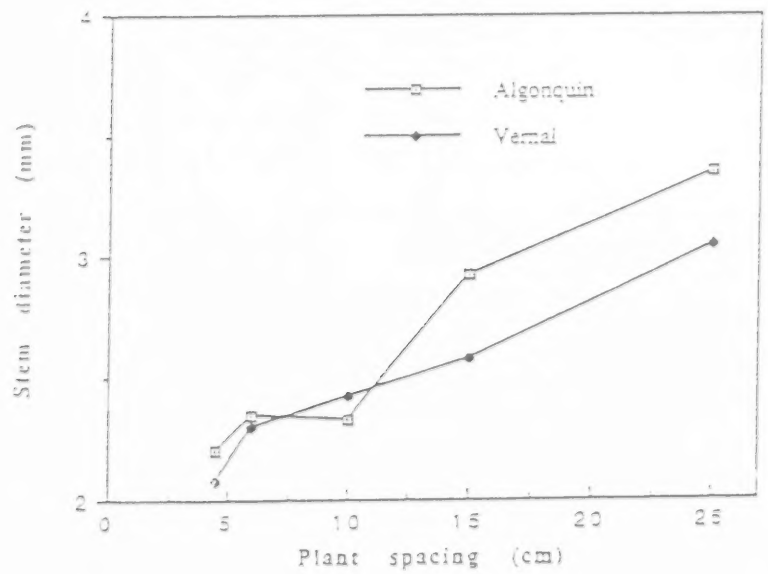


Figure 3.25. Effect of plant spacing on stem diameter in Algonquin and Vernal in the fall of establishment year of 1993.

($p<0.001$) linear effect of plant spacing on stem diameter in the fall harvest during the establishment year (Fig.3.25). The greatest stem diameter was attained at a 25 cm plant spacing and the least stem diameter at the 4.5 cm plant spacing in both cultivars. There was no significant effect of cultivar or cultivar x plant spacing interaction on stem diameter.

A significant ($p<0.001$) linear effect of plant spacing on stem diameter occurred at the first harvest, and a significant linear ($p<0.001$) and quadratic ($p<0.01$) relationship at the second harvest during the first production year of 1993. However, there was no significant effect of cultivar or cultivar x plant spacing interaction on stem diameter.

With successive harvests during the first production year there was very little change in stem diameter (Fig.3.26 and 3.27). The greatest stem diameter was attained at the 25 cm plant spacing and least was attained at the 4.5 cm plant spacing in each harvest for both cultivars.

3.3.4.4. Stem length.

There was a significant ($p<0.001$) linear and ($p<0.001$) quadratic effect of plant spacing on stem length in the fall harvest of 1992 (Fig.3.28). The stem length was greatest at a 15 cm plant spacing in Vernal, and a 25 cm plant spacing in Algonquin. For both cultivars stem length was the least at a 4.5 cm plant spacing. There were no cultivar effects or cultivar x plant spacing interaction on stem length. In alfalfa plants transplanted in 1993, there was a significant ($p<0.05$) linear effect of plant spacing on stem length in the fall harvest during the establishment year (Fig.3.29). In both cultivars, plants grown at a 4.5 cm plant spacing had the shortest stems, and the longest stems occurred at the 25 cm plant spacing in Algonquin, and at the 10 cm plant spacing in Vernal. There was no significant effect of cultivar or cultivar x plant spacing interaction on stem length.

In the first production year, there were significant ($p<0.001$)

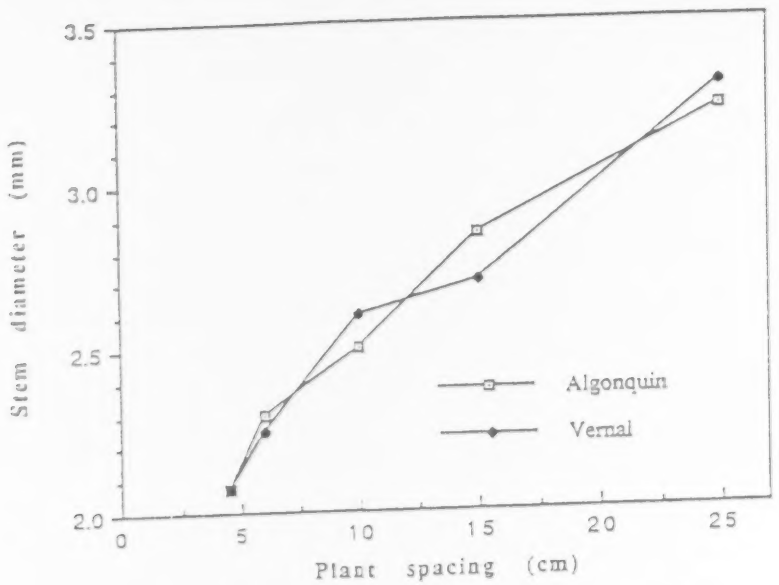


Figure 3.26. Effect of plant spacing on stem diameter in Algonquin and Vernal at the first harvest in the first production year of 1993.

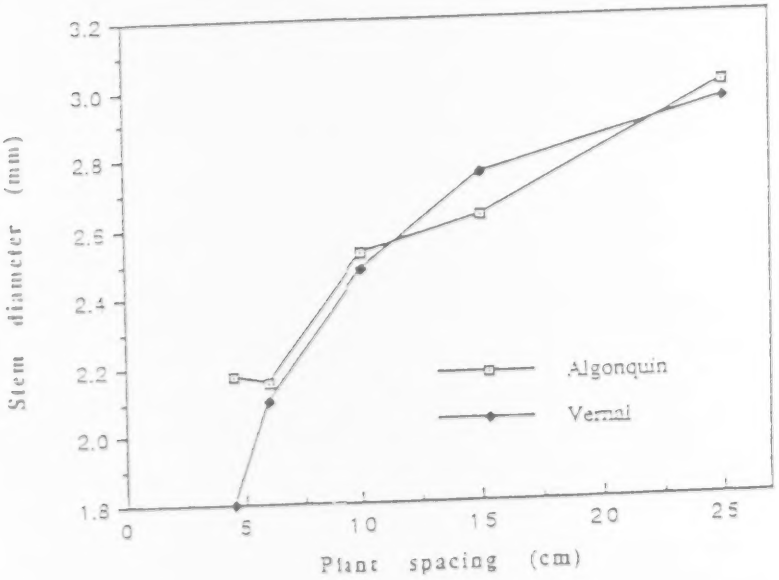


Figure 3.27. Effect of plant spacing on stem diameter in Algonquin and Vernal at the second harvest in the first production year of 1993.

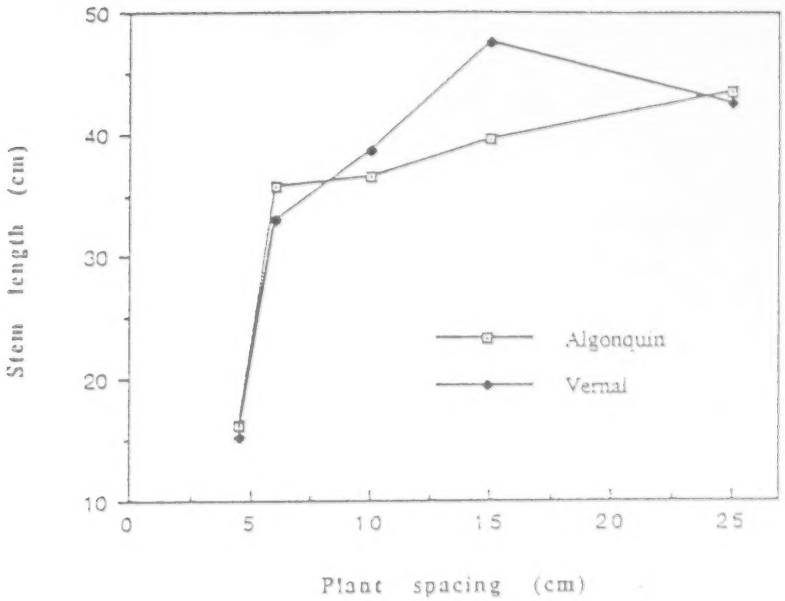


Figure 3.28. Effect of plant spacing on stem length in Algonquin and Vernal in the fall of establishment year of 1992.

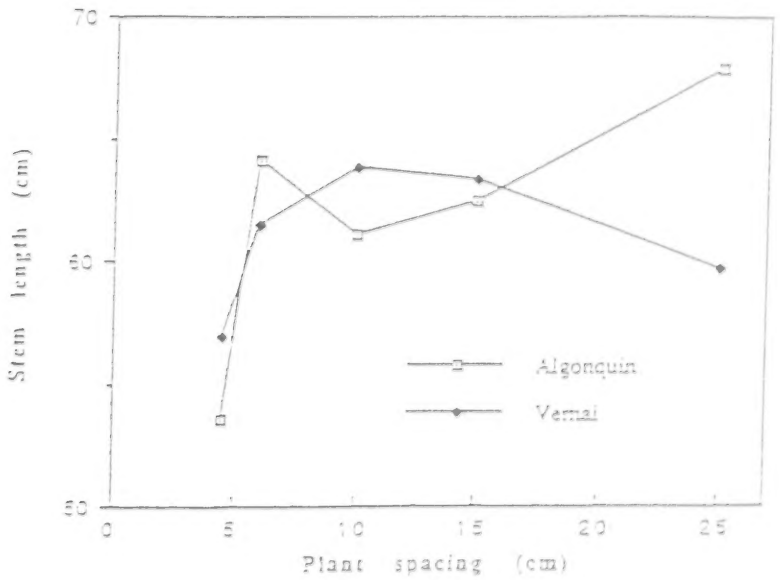


Figure 3.29. Effect of plant spacing on stem length in Algonquin and Vernal in the fall of establishment year of 1993.

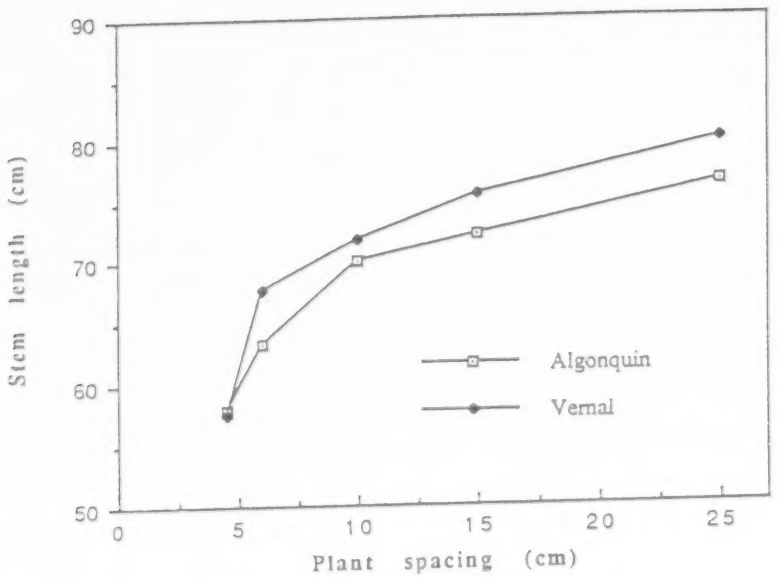


Figure 3.30. Effect of plant spacing on stem length in Algonquin and Vernal at the first harvest in the first production year of 1993.

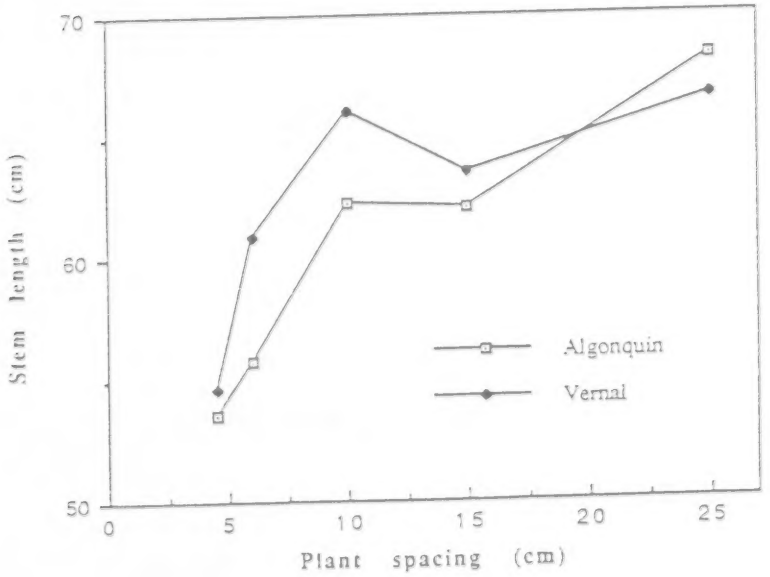


Figure 3.31. Effect of plant spacing on stem length in Algonquin and Vernal at the second harvest in the first production year of 1993.

linear and ($p<0.001$) quadratic effects of plant spacing on stem length at the first and second harvest (Fig.3.30-3.31). The greatest stem length was attained at the 25 cm plant spacing and the least at the 4.5 cm plant spacing in both cultivars with both harvests.

With successive harvests, the stem length declined. There were no significant cultivar effects or cultivar x plant spacing interaction on stem length at either harvest.

3.3.4.5. Nodes per stem.

There was a significant ($p<0.001$) linear and ($p<0.001$) quadratic effect of plant spacing on nodes per stem in the fall harvest of 1992 (Fig.3.32), but there was no cultivar effect or cultivar x plant spacing interaction. In alfalfa plants, transplanted in 1993, there was a significant ($p<0.001$) linear effect of plant spacing on the number of nodes per stem in the fall harvest during the establishment year. There was no significant effect of cultivar or cultivar x plant spacing interaction on number of nodes per stem.

After the first winter there was a significant ($p<0.05$) cultivar effect but no significant effect of plant spacing or cultivar x plant spacing interaction on number of nodes per stem at the first harvest during the first production year of 1993 (Fig.3.34). The difference in number of nodes per stem between cultivars was less than one node. At the second harvest, there was a significant ($p<0.001$) linear and ($p<0.05$) quadratic effect of plant spacing on nodes per stem. Thus, as plant spacing increased, the number of nodes per stem increased. The greatest number of nodes per stem occurred at the 25 cm plant spacing and the smallest number of nodes per stem occurred at the 4.5 cm plant spacing. There was no significant effect of cultivar or cultivar x plant spacing interaction at the second harvest in 1993 (Fig.3.35). The number of nodes per stem in the first harvest was greater than that of second harvest.

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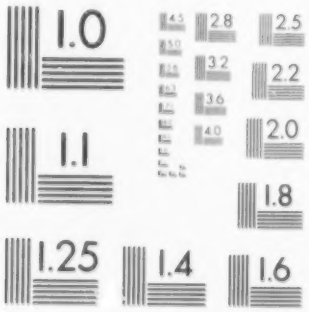

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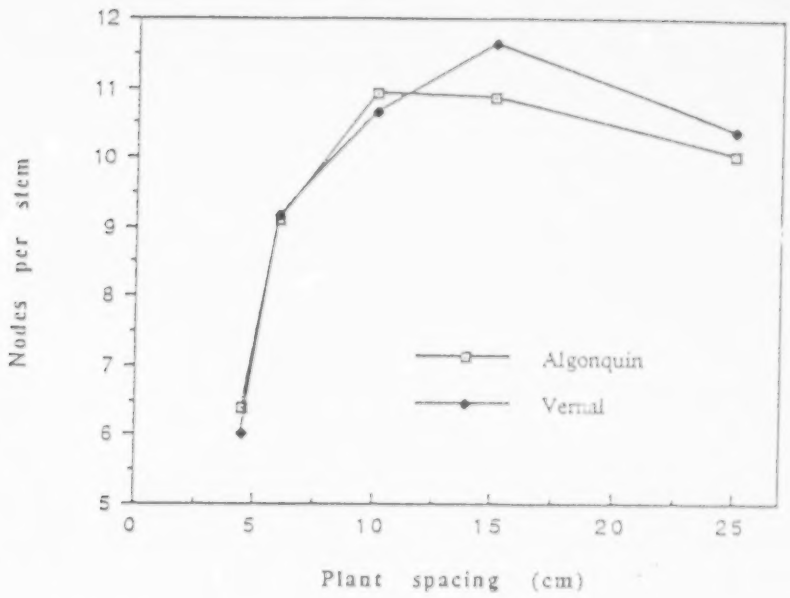

Figure 3.32. Effect of plant spacing on nodes per stem in Algonquin and Vernal in the fall of establishment year of 1992.

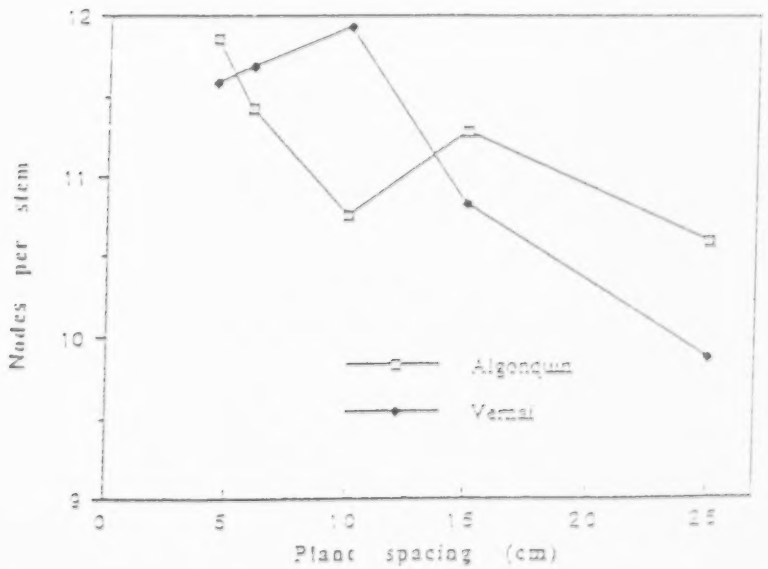

Figure 3.33. Effect of plant spacing on nodes per stem in Algonquin and Vernal in the fall of establishment year of 1993.

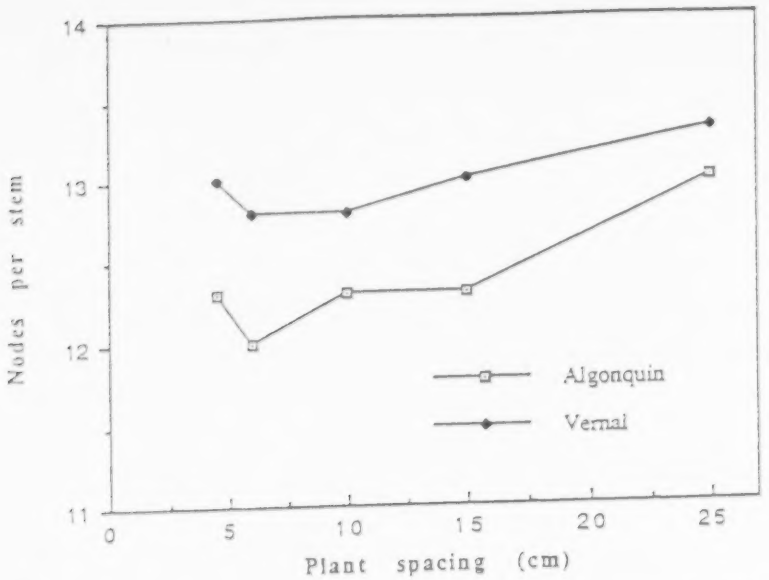

Figure 3.34. Effect of plant spacing on nodes per stem in Algonquin and Vernal at the first harvest in the first production year of 1993.

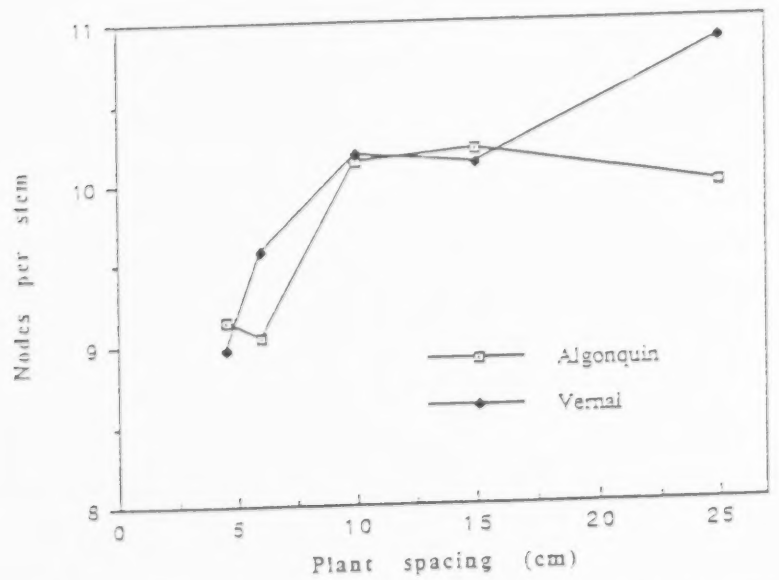

Figure 3.35. Effect of plant spacing on nodes per stem in Algonquin and Vernal at the second harvest in the first production year of 1993.

3.3.4.6. Leaf area per m^2.

In the fall harvest during the establishment year of 1992 there were significant ($p<0.05$) linear and ($p<0.001$) quadratic effects of plant spacing on leaf area per m^2. The transplanted plots which were at 6, 10, 15, and 25 cm plant spacings showed that the leaf area per m^2 decreased as plant spacing increased(Fig.3.36). There was no significant cultivar effect or cultivar x plant spacing interaction on leaf area per m^2. The leaf area per m^2 in the fall harvest of the establishment year of 1993 is given in Fig.3.37. There was a significant ($p<0.01$) linear relationship between plant spacing and leaf area per m^2. The greatest leaf area per m^2 occurred at a 6 cm plant spacing in Algonquin and Vernal. The lowest leaf area per m^2 was attained at a 25 cm plant spacing in both cultivars.

At the first and second harvests during the first production year of 1993, there was a significant ($p<0.001$) linear relationship between the plant spacing and leaf area per m^2. As plant spacing increased the leaf area per m^2 decreased (Fig.3.38-3.39). The greatest leaf area per m^2 occurred at the 6 cm plant spacing and the least leaf area per m^2 occurred at the 25 cm plant spacing. There was no significant cultivar effect or cultivar x plant spacing interaction.

3.3.4.7. Leaf to stem ratio.

As plant spacing increased the leaf to stem ratio decreased in each cultivar in the fall of 1992 (Fig.3.40). There was a significant ($p<0.001$) linear effect of plant spacing on leaf to stem ratio. The highest leaf to stem ratio was attained at a 4.5 cm plant spacing and the least ratio was attained for plants grown at a 25 cm plant spacing for both cultivars. There was no significant cultivar effect or cultivar x plant spacing interaction on leaf to stem ratio. In alfalfa plants, transplanted in 1993, there was a significant ($p<0.01$) cultivar effect and Algonquin had a higher L/S ratio than Vernal in the fall harvest during the establishment year. However, there was no significant effect of plant spacing or cultivar x plant spacing interaction on leaf

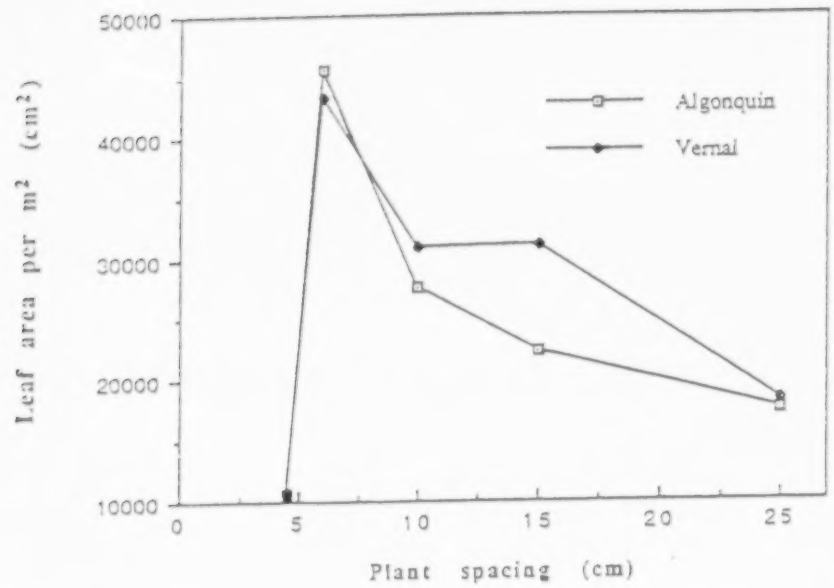

Figure 3.36. Effect of plant spacing on leaf area per m^2 in Algonquin and Vernal in the fall of establishment year of 1992.

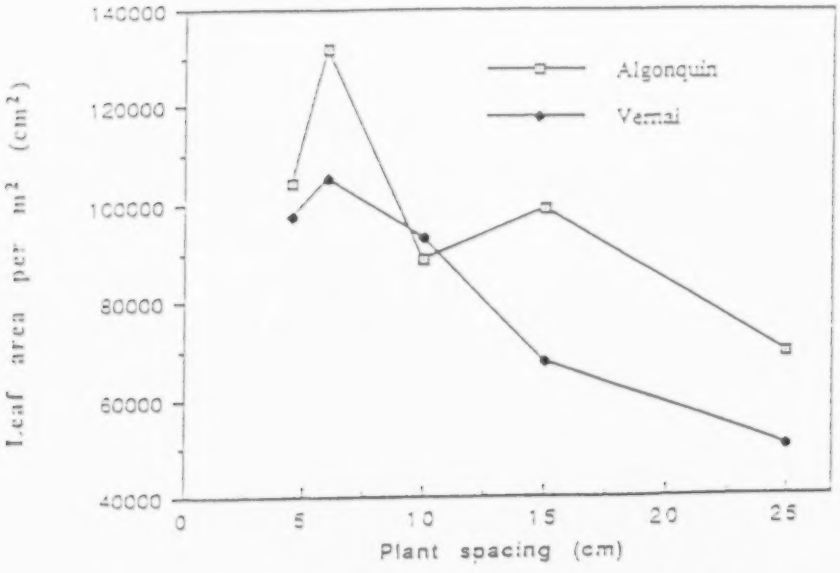

Figure 3.37. Effect of plant spacing on leaf area per m^2 in Algonquin and Vernal in the fall of establishment year of 1993.

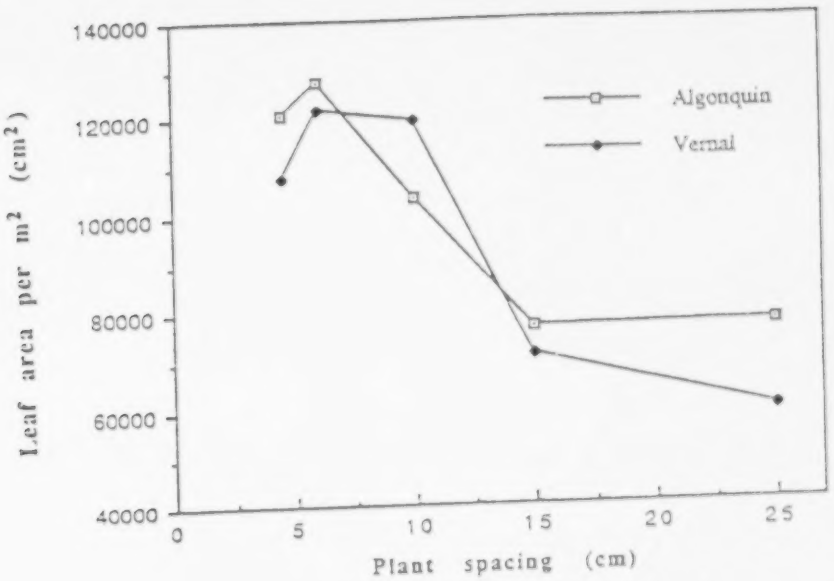

Figure 3.38. Effect of plant spacing on leaf area per m^2 in Algonquin and Vernal at the first harvest in the first production year of 1993.

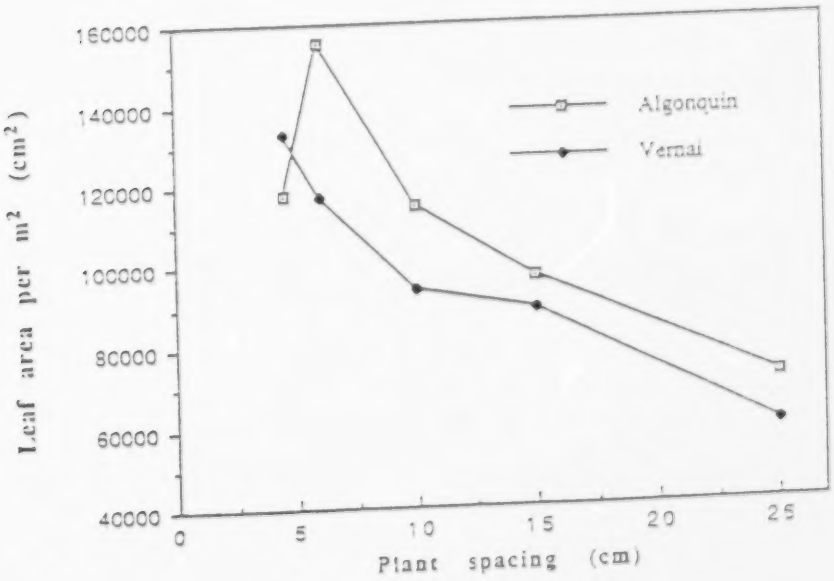

Figure 3.39. Effect of plant spacing on leaf area per m^2 in Algonquin and Vernal at the second harvest in the first production year of 1993.

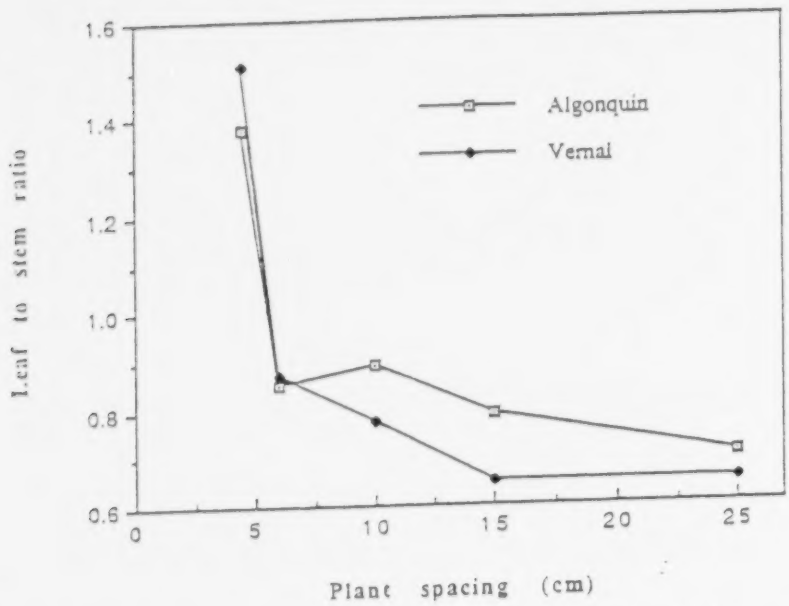

Figure 3.40. Effect of plant spacing on leaf to stem ratio in Algonquin and Vernal in the fall of establishment year of 1992.

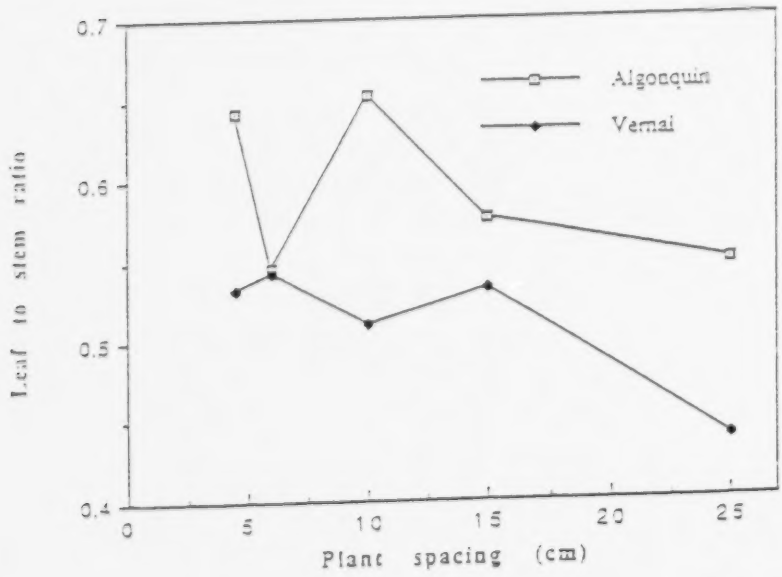

Figure 3.41. Effect of plant spacing on leaf to stem ratio in Algonquin and Vernal in the fall of establishment year of 1993.

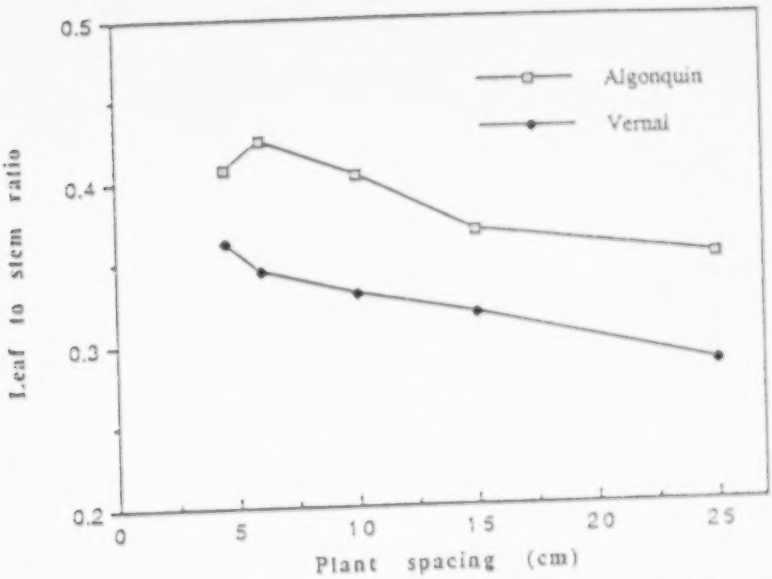

Figure 3.42. Effect of plant spacing on leaf to stem ratio in Algonquin and Vernal at the first harvest in the first production year of 1993.

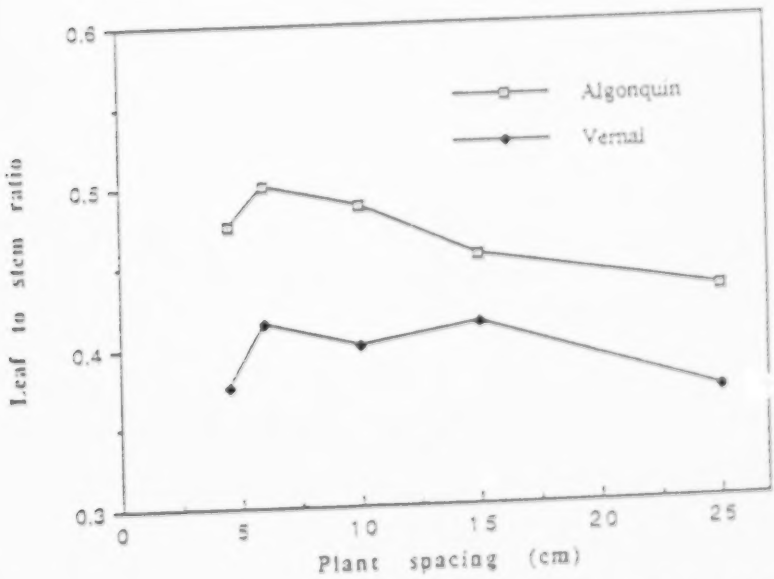

Figure 3.43. Effect of plant spacing on leaf to stem ratio in Algonquin and Vernal at the second harvest in the first production year of 1993.

to stem ratio (Fig.3.41).

In the first harvest during the first production year of 1993 there was a significant ($p<0.001$) cultivar effect on the leaf to stem ratio, with the ratio of Algonquin being somewhat higher than that of Vernal (Fig.3.42). As plant spacing increased the leaf to stem ratio generally decreased in both cultivars resulting in a significant ($p<0.01$) linear effect of plant spacing on leaf to stem ratio. There was no significant cultivar x plant spacing interaction on leaf to stem ratio.

At the second harvest there was a significant ($p<0.001$) cultivar effect on leaf to stem ratio (Fig.3.43) and no significant plant spacing or cultivar x plant spacing interaction. The leaf to stem ratio was a little higher in Algonquin than in Vernal. The L/S ratio at the second harvest was a little higher than that of the first harvest.

3.3.5. Quality

3.3.5.1. Crude protein (C.P.).

At the fall harvest of the establishment year of 1992 there was no significant effect of cultivar, plant spacing, or cultivar x plant spacing interaction on the C.P. content (Fig.3.44).

Unlike the establishment year, during the first production year of 1993 there was a significant ($p<0.05$) difference between cultivars. The cultivar Algonquin contained somewhat higher C.P. than Vernal. In the first production year there was also a significant ($p<0.05$) quadratic effect of plant spacing on C.P at the first harvest. The greatest C.P. (17.4 %) for Algonquin occurred at the 10 cm plant spacing and the lowest C.P. (15.6 %) at the 25 cm plant spacing. The crude protein of Vernal alfalfa was also lowest at the 25 cm plant spacing (Fig.3.45).

At the second harvest there was a significant ($p<0.05$) cultivar effect and significant ($p<0.05$) cultivar x plant spacing interaction. Similar to the first harvest, the C.P. of Algonquin was a little higher than that of Vernal at most densities (Fig.3.46). There was no

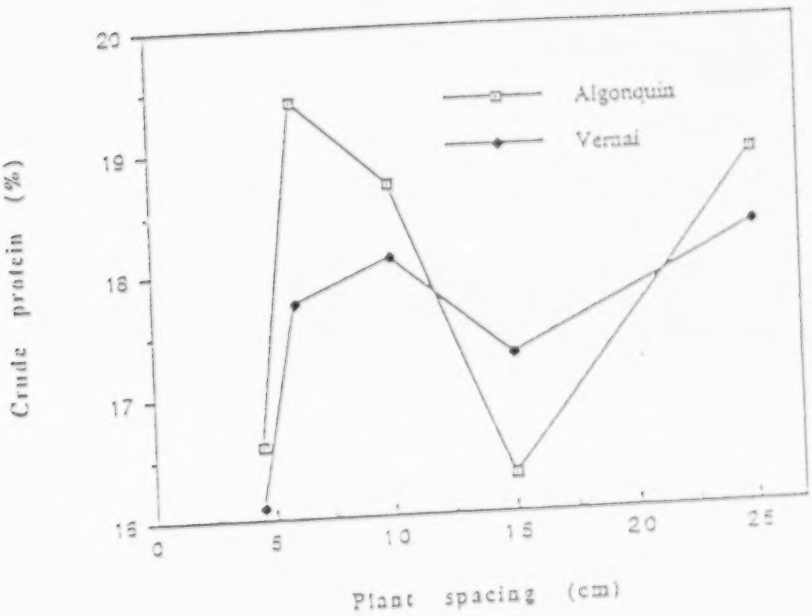

Figure 3.44. Effect of plant spacing on crude protein in Algonquin and Vernal in the fall of establishment year of 1992.

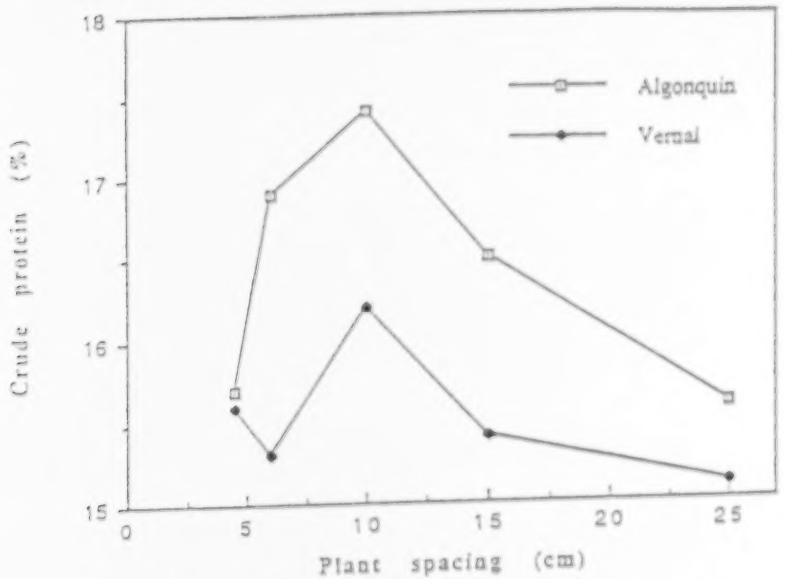

Figure 3.45. Effect of plant spacing on crude protein in Algonquin and Vernal at the first harvest in the first production year of 1993.

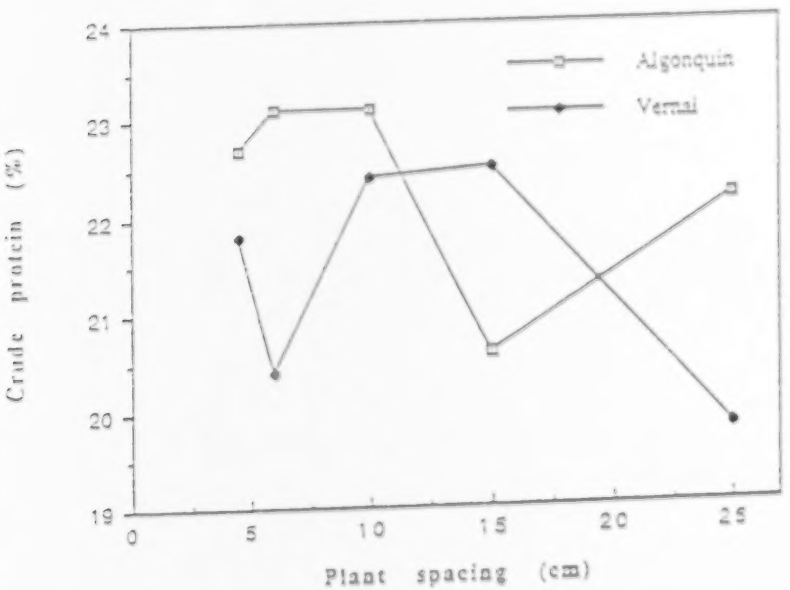

Figure 3.46. Effect of plant spacing on crude protein in Algonquin and Vernal at the second harvest in the first production year of 1993.

significant effect of plant spacing on C.P. at the second harvest.

3.3.5.2. Acid Detergent Fiber (ADF).

There was a significant ($p<0.05$) effect of plant spacing on ADF in the fall of 1992. The greatest ADF occurred at a 4.5 cm plant spacing in Algonquin and a 15 cm plant spacing in Vernal. The lowest ADF was attained at a 6 cm plant spacing in Algonquin and a 25 cm plant spacing in Vernal (Fig.3.47). However, there was no cultivar effect or cultivar x plant spacing interaction.

At the first harvest in the first production year of 1993, there was a significant ($p<0.05$) difference between the cultivars for ADF. Algonquin had a lower ADF (38.2 %) than Vernal (39.7 %) (Fig.3.48). There was no significant effect of plant spacing or cultivar x plant spacing interaction on ADF. The ADF range was 39-42 % at the first harvest.

At the second harvest, there was a significant ($p<0.01$) cultivar effect and Vernal had a higher mean ADF (38.3 %) than Algonquin (35.3 %) (Fig.3.49). There was no significant effect of plant spacing or cultivar x plant spacing interaction on ADF. The ADF content at the second harvest (i.e., average of 36.8 %) was a little lower than at the first harvest (i.e., average of 38.9 %) at all plant spacings.

3.3.5.3. Neutral Detergent Fiber (NDF).

Similar to the trend for ADF, there was a significant ($p<0.05$) effect of plant spacing on NDF at the fall harvest of the establishment year, 1992. The greatest NDF occurred at a 4.5 cm plant spacing in both cultivars, and the lowest NDF was attained at a 25 cm plant spacing in Algonquin and a 6 cm plant spacing in Vernal (Fig.3.50).

In the first production year, 1993, there was a significant ($p<0.05$) cultivar effect and Algonquin had lower NDF (47.5 %) than Vernal (49.1 %) at the first harvest (Fig.3.51). But there was no significant effect of plant spacing or cultivar x plant spacing

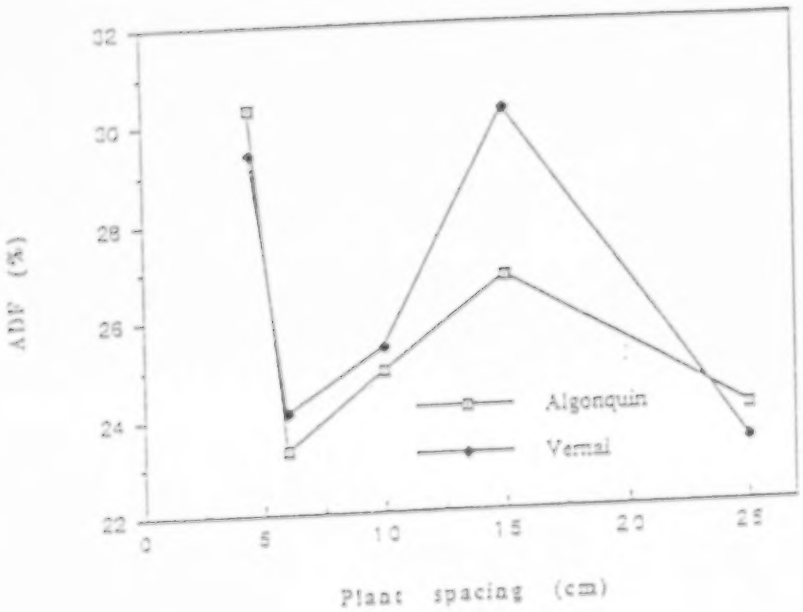

Figure 3.47. Effect of plant spacing on ADF in Algonquin and Vernal in the fall of establishment year of 1992.

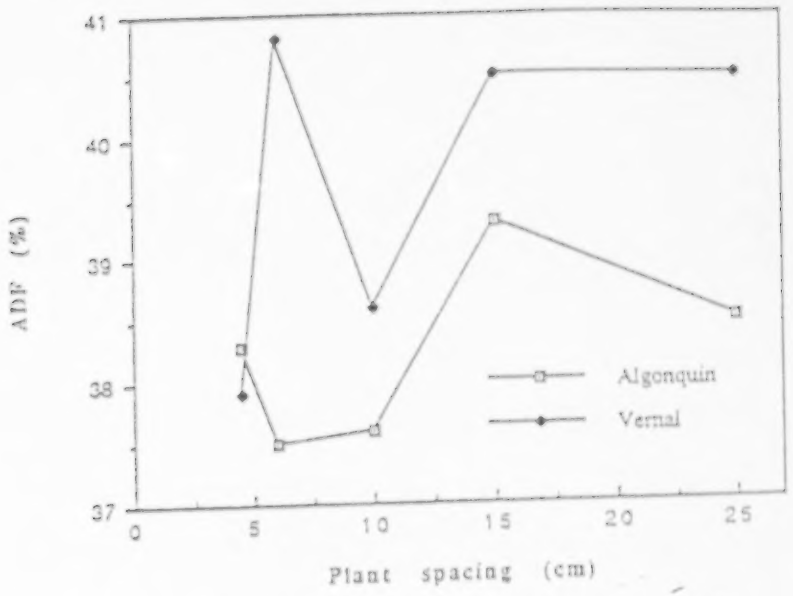

Figure 3.48. Effect of plant spacing on ADF in Algonquin and Vernal at the first harvest in the first production year of 1993.

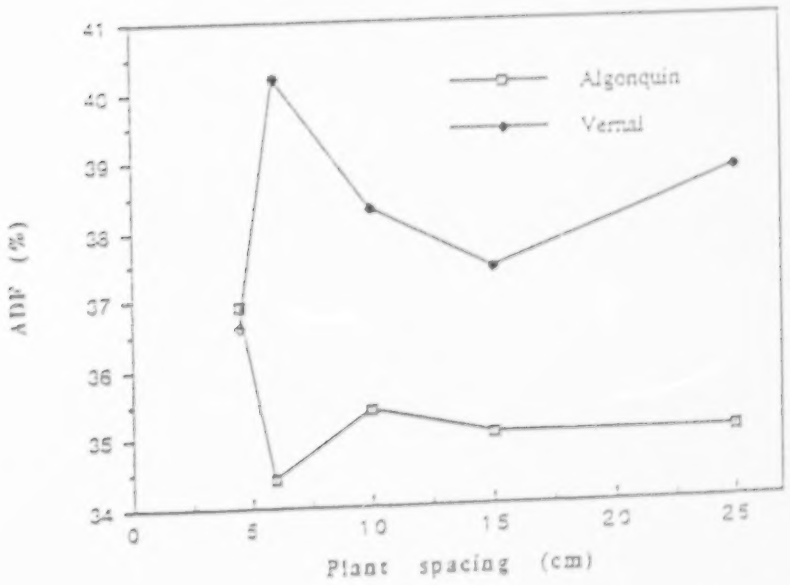

Figure 3.49. Effect of plant spacing on ADF in Algonquin and Vernal at the second harvest in the first production year of 1993.

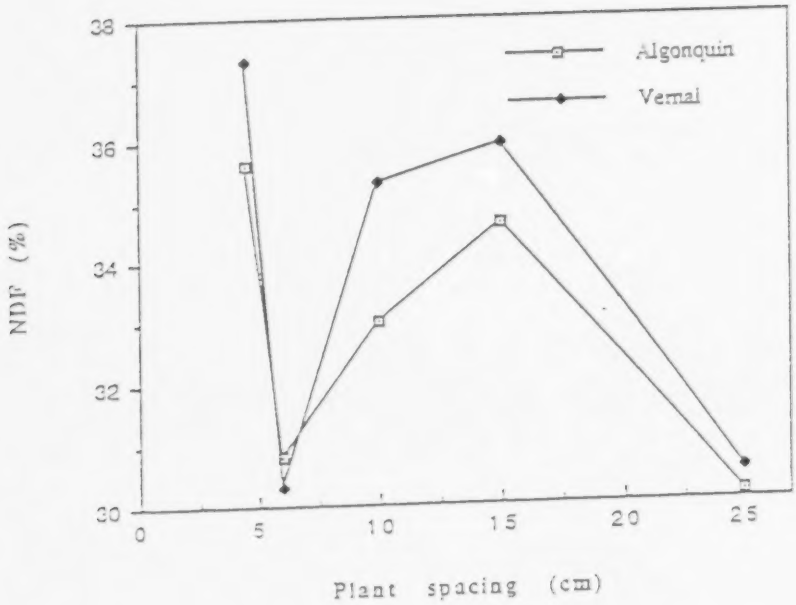

Figure 3.50. Effect of plant spacing on NDF in Algonquin and Vernal in the fall of establishment year of 1992.

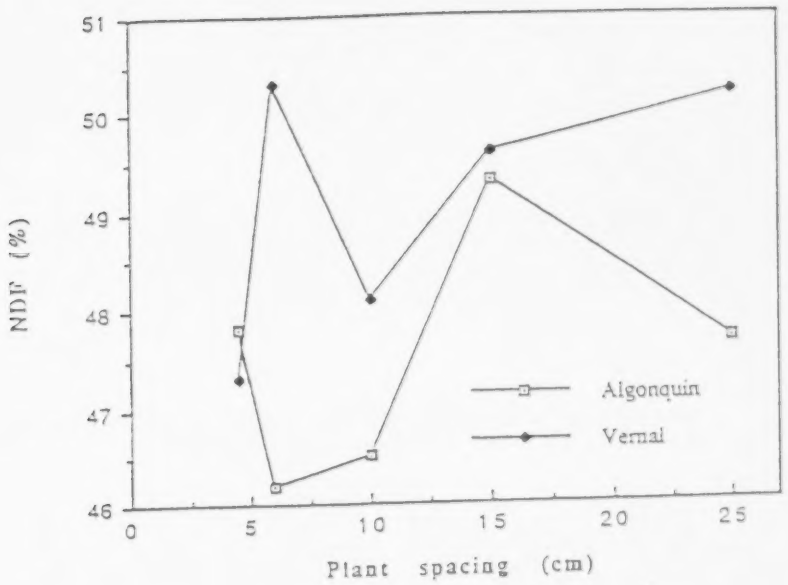

Figure 3.51. Effect of plant spacing on NDF in Algonquin and Vernal at the first harvest in the first production year of 1993.

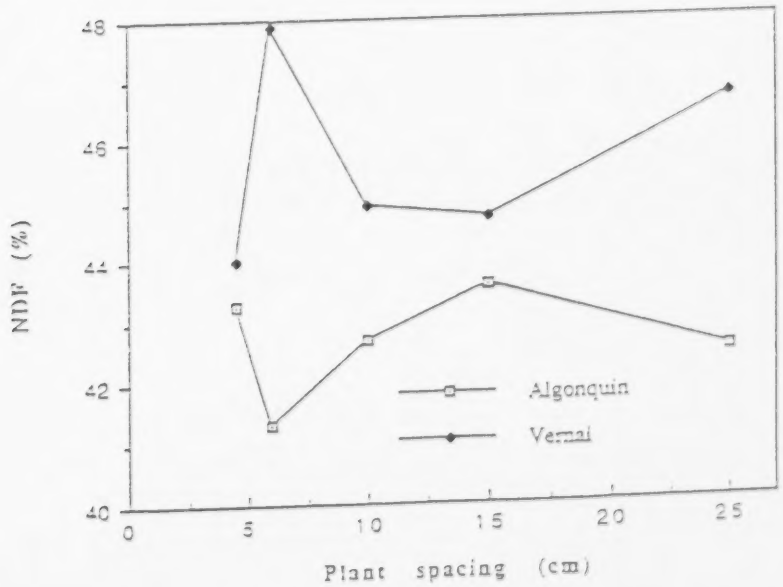

Figure 3.52. Effect of plant spacing on NDF in Algonquin and Vernal at the second harvest in the first production year of 1993.

interaction on NDF. The NDF range was between 47 - 50 % at the first harvest.

At the second harvest there was a significant ($p<0.001$) difference in cultivars and Vernal had higher NDF (45.6 %) than Algonquin (42.6 %) (Fig.3.52). However, there was no significant effect of plant spacing or cultivar x plant spacing interaction on NDF. The NDF range was between 41 and 48 % at the second harvest. The mean NDF content at the second harvest (i.e., 44.1 %) was a little lower than at the first harvest (i.e., 48.3 %) at all plant spacings.

3.4. Discussion

Stand density is one of the factors that determines yield and persistence of alfalfa. However, the optimum stand density varies with climate and soil conditions. As establishment costs increase, the question arises whether present seeding rate or population densities are optimum for obtaining maximum yield and quality.

Yield of alfalfa (*Medicago sativa* L) can be described by three yield components: plants per area, shoots per plant, and yield per shoot (Volenec et al, 1987). During the seeding year of the alfalfa plants transplanted in 1992, plants grown at the highest stand density (494 plants m^{-2}) had a lower yield because they were younger and smaller than plants at other stand densities. However, in the seeding year 1993, as plant spacing increased, yield was stand density-dependent (Fig.3.2). Although plants grown at a 25 cm spacing had more shoots per plant and a higher yield per shoot than plants at other plant spacings, these increases could not compensate for the low population density. The difference in yield in the fall harvest during the establishment year 1993 between 4.5 and 25 cm plant spacings was 1.6 ton ha^{-1} even though 31 times as many plants were transplanted at a 4.5 cm plant spacing compared to 25 cm plant spacing. The difference in yield at the high stand density in the establishment years of 1992 and 1993, might be attributable to the earlier seeding date in 1993. Thus, when alfalfa plants grown at a 4.5 cm plant spacing were seeded earlier in the season and harvested at the same growth stage as the other transplanted plants, yield in the establishment year was density-dependent.

In the first production year, the annual total yield from alfalfa plants grown at a high population density was greater than that of a low population density. Although plants grown at a low stand density had more shoots per plant (Fig.3.18-3.19), a higher yield per shoot (Fig.3.22-3.23) and a better winter survival (Fig.3.6) than those at a high population density, all these factors combined could not compensate for fewer plants per unit area at the low population density. This indicated that good winter survival, resulting in more surviving plants per unit area, was able to compensate for lower

shoots per plant and yield per shoot at a high population density. The higher yield of Vernal is partly attributed to its better winter survival although overall there was a low correlation between winter survival and yield. Similarly, Bolger and Meyer (1983) in North Dakota, USA found that forage yield at the highest plant density was not significantly greater than that of the lower densities.

It was hypothesized that winter kill would be higher in the first winter after seeding at the high stand densities due to the plants small root and crown size. However, even at the high population densities, winter survival in 1992-1993 was very high (Fig.3.6) even though the monthly minimum temperature (Fig.a.2) was lower during the winter of 1992-1993 than during 1991-1992. The temperature during the winter of 1992-1993 was similar to the 30 year average. This suggests that although plants with small roots and crown are generally more susceptible to winter kill, it is not always true. In Bolger and Meyer's study (1983), 24 to 36 % of the plants at the 22 to 43 plants m^{-2} densities, and 10 % of the plants at the 11 plants m^{-2} density failed to survive. Van Keuren (1973) reported that after the first winter, different seeding rates had similar plant density counts in North Dakota, USA. In this study, less than 5 % of the plants at the low population densities (i.e., 16 and 45 plants m^{-2}) failed to survive their first winter, and less than 7 % of the plants at the high population densities (i.e., 278 and 494 plants m^{-2}) failed to survive. In both Vernal and Algonquin, winter survival was maximized at a population of 45 plants m^{-2} or less.

When TNC was expressed as grams of TNC per root, plants at the 25 cm spacing contained higher levels of TNC than plants at a 4.5 cm plant spacing. This was because the root size declined linearly from the 25 cm to the 4.5 cm plant spacing. Thus, if the total amount of TNC per root affects winter survival, it would be expected that plants with larger roots at the low population densities would survive better than those at high population densities. These findings are consistent with the previous work of Sund and Barrington (1976). The grains of TNC per root measured in the spring of 1993 had declined compared to the previous fall. This indicates that carbohydrate reserves were used for plant metabolic activities

during the winter and to sustain winter survival.

The higher TEG in plants sampled in the fall compared to those sampled in the spring after winter indicates that carbohydrate reserves were used during winter to survive winter weather conditions. Since plants grown at a 25 cm plant spacing had the largest root mass, their TEG was lowest in spite of higher etiolated shoot weight. However, in terms of absolute etiolated shoot dry weight, plants grown at a 25 cm plant spacing produced more grams of etiolated shoot than at the other spacings. Thus, TEG results reflect the same trends as grams of TNC.

The minimum and maximum LT 50 occurred at the lowest- and highest plant density, respectively. This suggests that larger alfalfa plants having more grams of TNC tolerate cold temperature stress better than smaller alfalfa plants.

Plants grown at the high population density had fewer shoots per plant and yield per shoot than those grown at a low population density. This is partly attributable to a smaller root and crown size resulting from intra-specific competition for light, moisture, and nutrients. As plant spacing increased, shoots per plant increased presumably due to less intra-specific competition resulting in more resources for each plant. These findings are consistent with previous studies (Bolger and Meyer, 1983; Rumbaugh 1963; Volenec et al., 1987).

The smaller stem diameter recorded at high plant densities is partly due to intra-specific competition for light. At a high population density the basal leaves are heavily shaded resulting in a lower photosynthetic capability, and a smaller shoot biomass than for plants at a low population density. Volenec et al (1987) reported that stem diameter decreased significantly as plant population increased. An alfalfa seeding rate study carried out in Oregon, USA, (McGuire,1981) showed that stem diameter and weight decreased as the season advanced for each harvest for the cultivars of Vernal and DuPuits. It was also shown that larger stemmed cultivars retained their large stem size, regardless of seeding rate and row spacing. This suggests that stem diameter is a genotype specific trait and not affected by seeding rate.

Trends in stem diameter, stem length, number of nodes per stem, leaf area m^{-2} and L/S ratio trends did not follow the yield trends at each harvest. This resulted in very low correlations between yield and each of these parameters and indicates that yield was not directly affected by stem diameter, stem length, or number of nodes per stem, leaf area m^{-2}, or L/S ratio and yield.

In the first production year there was a significant difference in C.P. between the cultivars Vernal and Algonquin. Algonquin had a higher percent of crude protein at each harvest (Fig.3.45-3.46). This was consistent with the trend of the leaf to stem ratio, with Algonquin having a higher leaf to stem ratio than Vernal. However, percent C.P. showed very low correlations with the leaf to stem ratio at each harvest. This suggests that % C.P. is genotype-dependent and is not greatly influenced by population density. The C.P. content at the second harvest was higher than at the first harvest. This might be attributed to the differences in L/S ratio. According to Kreuger and Hansen (1974) in South Dakota, during the seeding year and the first production year, the percent of C.P. in alfalfa was not affected by the seeding rate even though alfalfa stems were finer at the higher seeding rates. McGuire (1981) also reported that in Ohio, the C.P. content was not influenced by the seeding rate. In this study, although C.P. of stems alone was not measured, the finer shoots produced at high population densities didn't have higher C.P. contents cpmpared to those from low population densities.

Acid detergent fiber (ADF) includes cellulose and lignin content which affects digestibility. Generally, as ADF increases the digestibility decreases. In this study, there was a significant cultivar difference in ADF at each harvest in the first production year of 1993, with Algonquin having a lower ADF content (Fig.3.45-3.46). Algonquin had a higher L/S ratio than Vernal at each harvest (Fig.3.42-3.43) and for each plant spacing, therefore the differences in ADF appeared to be genetic rather than environmentally controlled effects. This agrees with Bolger and Meyer's results (1983), who reported no significant effect of plant population on concentrations of C.P. or ADF in alfalfa. Sund and Barrington (1976) also reported from Wisconsin, USA that seeding rates of 6.7 to 40.4

kg ha^{-1} did not influence yield or cell wall constituents (CWC), ADF, or acid detergent lignin (ADL). There were low negative correlations between the L/S ratio and ADF.

At the first harvest during the first production year of 1993 NDF differed between cultivars, Vernal having a higher NDF than Algonquin (Fig.3.51). This result was opposite to the leaf to stem ratio results with Algonquin having a higher L/S ratio than Vernal at each harvest (Fig.3.42-3.43). This suggests that the high L/S ratio in Algonquin resulted in lower NDF contents (hemi-cellulose, cellulose, and lignin). These findings are consistent with previous studies (Meyer 1985; Van Keuren 1973; Cherney et al.1986). The NDF at the second harvest was somewhat lower (Fig.3.52) than at the first harvest, while the L/S ratio was somewhat higher. These difference between the first and second harvest might be partly attributable to differences in origin of stems. Nelson and Smith (1968) reported that stems from crown buds in spring growth have a low L/S ratio compared to stems from axillary basal buds in regrowth.

In this study, over a period of two years high stand densities (278 plants m^{-2} or more) appeared to be of little advantage in terms of increasing winter survival, yield, or forage quality compared to low population densities (45 plants m^{-2} or less).

3.5. Conclusions.

Total annual yield was greatest for plants grown at a high plant density (494 plants m^{-2}) in Algonquin and a moderate plant density (45 plants m^{-2}) in Vernal, and the least for a 25 cm plant spacing (16 plants m^{-2}) in both cultivars in the first production year. However, at the highest plant density, annual total yield was only 0.4 ton ha^{-1} more in Vernal and 2.5 ton ha^{-1} more in Algonquin than at the lowest plant density in the first production year.

Winter survival was maximized at a plant population of 45 plants m^{-2} or less. The grams of TNC per root increased and TEG decreased as the stand density decreased. The lowest LT 50 occurred at the lowest stand density (16 plants m^{-2}) and the highest LT 50 was attained at the highest stand density (494 plants m^{-2}).

The number of shoots per plant, yield per shoot, and stem diameter increased linearly with decreasing population density. However, the other morphological characteristics, stem length, node number per stem, leaf area per m^{-2}, and leaf to stem ratio showed variable responses.

C.P. was affected by the density quadratically showing the highest C.P. at a 10 cm plant spacing in the first harvest and there was no effect of plant density at the second harvest in the first production year. ADF and NDF were not affected by the population density. Cultivar Algonquin had higher C.P., and lower ADF and NDF contents than Vernal.

From this study, high stand density appeared to be of little economic advantage compared to low stand density in terms of winter survival, yield and quality.

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Chapter 4. General Discussion and Conclusions.

4.1. Discussion.

At a high population density the major factor affecting yield was the number of plants. A high level of winter survival could compensate for fewer shoots per plant and lower yield per shoot, whereas, at a low population density the major factor was high shoot number per plant and yield per shoot, which could compensate for fewer plants per unit area. Thus, where winter survival was high at a high population density, yield exceeded that of a low population density.

Although 25 times more plants were transplanted at a 6 cm plant spacing compared to the 30 cm plant spacing, there was only a 1.2 ton ha^{-1} difference in the second production year compared to 7.0 ton ha^{-1} in the first production year. As well, the difference in annual total yield between a 4.5 cm and a 25 cm plant spacing was only 1.5 ton ha^{-1} in alfalfa plots in the first production year. This suggested that a high seeding rate gave very little economic advantage.

There were cultivar differences in annual total yield at a range of plant densities. In this study, the cultivar difference was due to better winter survival of Vernal than Algonquin. This suggests that selecting a winter hardy cultivar would result in a higher productivity.

Although there were very low correlations between winter survival and measures of parameters thought to be associated with cold hardiness (TNC, TEG, and LT 50), the large alfalfa plants had a higher survival rate than smaller plants. There were few or no dead plants at populations of 45 or 11 plants m^{-2}.

The number of shoots per plant and yield per shoot increased linearly with decreasing plant population densities. This is attributed to a smaller root and crown size resulting from intra-specific competition for light, moisture, and nutrients at high plant densities.

Crude protein increased to a 10 cm plant spacing and then decreased with increasing plant spacing, whereas, ADF, and NDF

content was not affected by the plant spacing. In this study, there was a cultivar difference, with Algonquin having higher C.P., and lower ADF and NDF than Vernal. These results might be attributed to the higher L/S ratio in Algonquin than in Vernal. Thus, selecting a cultivar with high L/S ratio may increase quality. Several researchers reported no significant effect of plant population density on quality parameters (Bolger and Meyer 1983; Meyer 1985; McGuire 1981).

In these trials, shoots per plant and yield per shoot were influenced by stand density. These factors in combination with winter survival determined the yield.

4.2. Conclusions.

1. High stand densities appeared to have little economic advantage over low population densities in terms of yield over a density range of 11 to 494 plants m^{-2}.

2. The winter survival was higher at a low population density with few or no dead plants at populations of 45 plants m^{-2} or less. Thus, winter survival is greater in large plants.

3. The weight of TNC per root increased and TEG decreased as the stand density decreased. The lowest LT 50 was associated with the lowest stand density (16 plants m^{-2}) whereas the highest LT 50 was associated with the highest stand density (278.plants m^{-2}). However, there were very low correlations between winter survival and cold hardiness parameters (TNC, TEG, and LT 50).

4. The number of shoots per plant and yield per shoot increased linearly with increasing plant spacing. However, the other morphological characteristics, stem diameter, stem length, number of nodes per stem, leaf area m^{-2}, and L/S ratio, had responses that were more variable.

5. Crude protein increased to the 10 cm plant spacing and then decreased with increasing plant spacing, whereas ADF and NDF content were not affected. There were cultivar differences with Algonquin having a higher C.P. content, and lower ADF and NDF content than Vernal.

In this study, high stand densities (278 plants m^{-2} or more) appeared to be of little economic advantage in terms of winter survival, yield, and forage quality. It will be necessary to continue this study to ascertain the long term effects of stand density on productivity, persistence, and quality of alfalfa.

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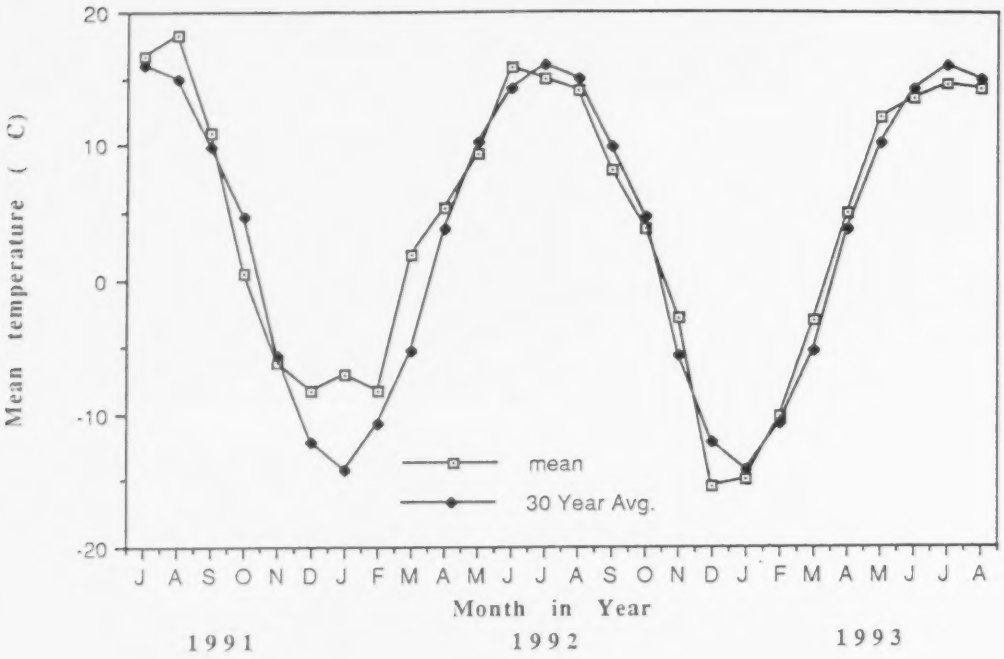

Figure a.1. Average monthly temperatures ((maximum + minimum) / 2) for the years 1991, 1992, 1993 and the 30 year average (1959 to 1990).

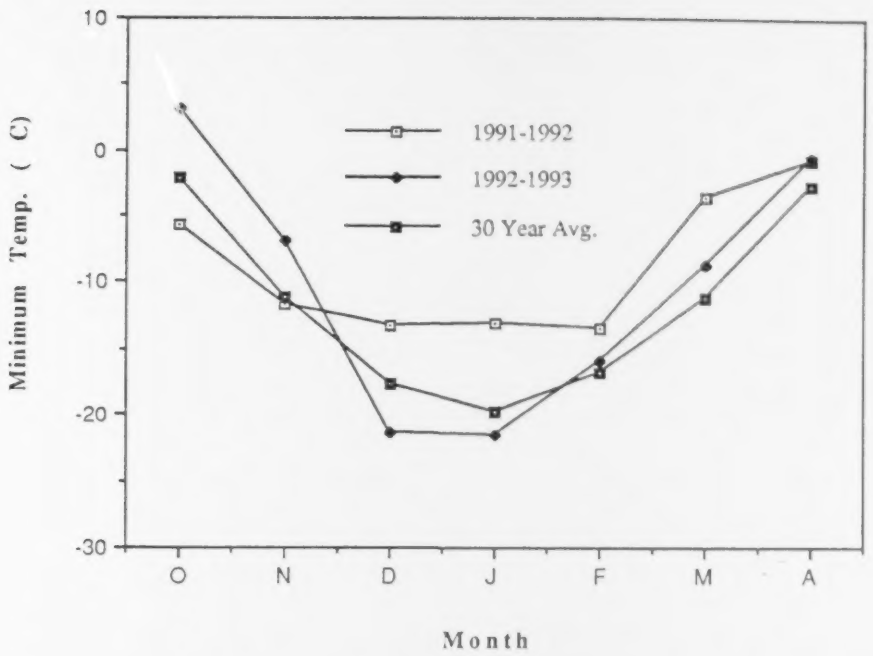

Figure a.2. Monthly minimum temperatures during the winter period for the years 1991, 1992, 1993, and the 30 year average (1959 to 1990).

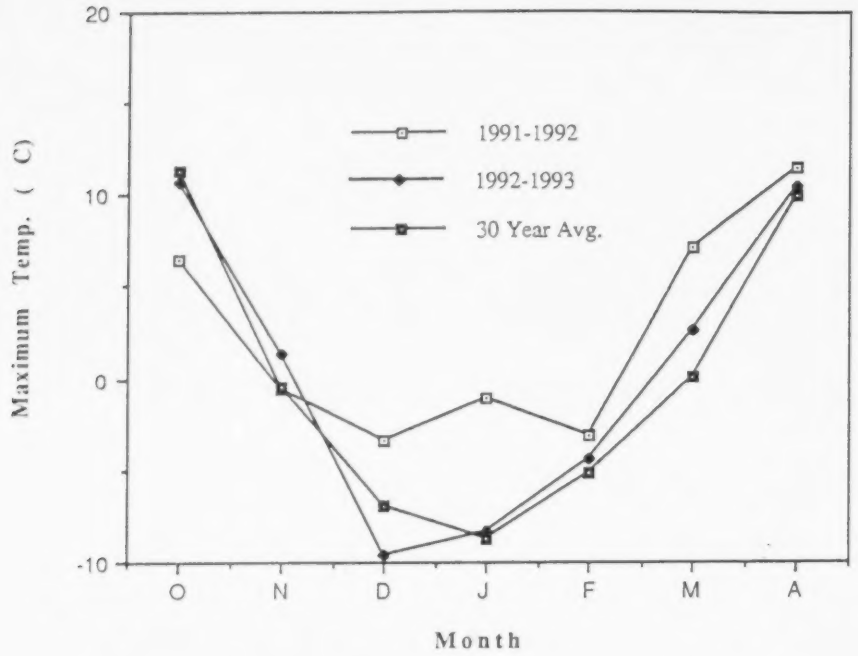

Figure a.3. Monthly maximum temperatures during the winter period for the years 1991, 1992, 1993, and the 30 year average (1959 to 1990).

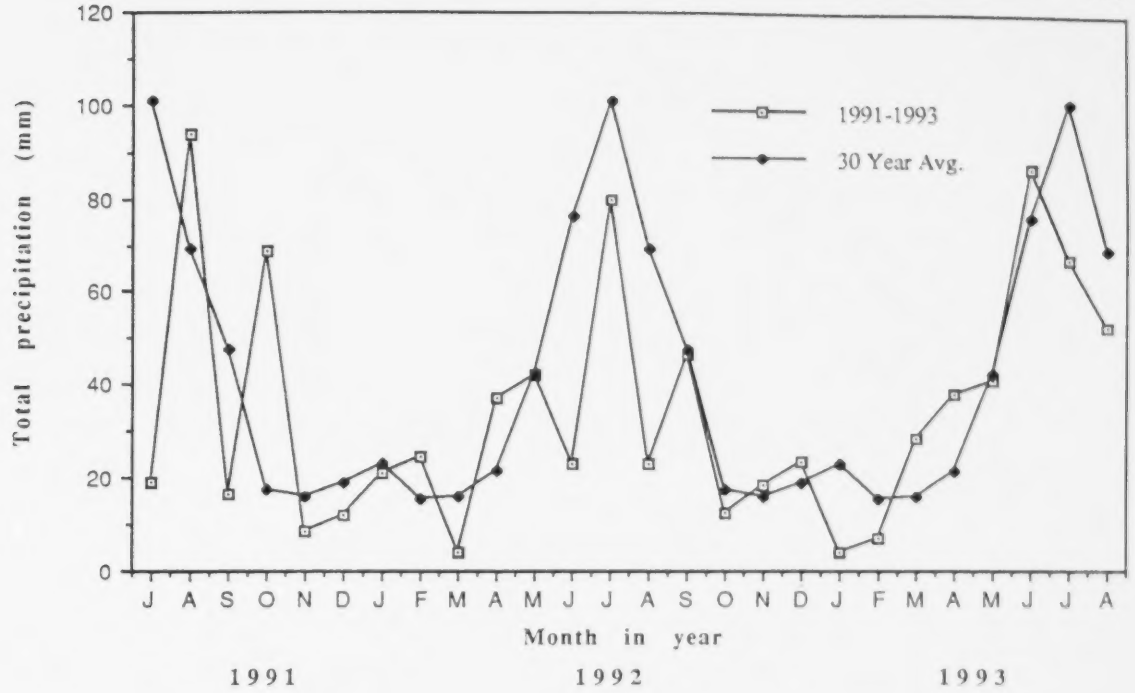

Figure a.4. Total monthly precipitation for the years 1991, 1992, 1993, and the 30 year average (1959 to 1990).

Table 1.1. Correlations between yield and winter survival, shoot number per plant, yield per shoot, stem diameter, stem lenght, nodes per stem, and leaf area in the first production year, 1992.

Parameters	Annual yield	1st harvest	2nd harvest	3rd harvest
Winter survival	-0.09	-	-	-
Shoots per plant	-	-0.3	-0.23	0.27
Yield per shoot	-	0.66	0.88	-0.21
Stem diameter	-	-0.05	0.13	0.07
Stem length	-	0.12	0.01	0.19
Nodes per stem	-	0.01	0.04	0.13
Leaf area	-	-0.09	0.26	-0.07

Table 1.2. Correlations between yield and shoot number per plant, yield per shoot, stem diameter, stem lenght, nodes per stem, and leaf area in the second production year, 1993.

Parameters	1st harvest	2nd harvest
Shoots per plant	0.16	-0.38
Yield per shoot	-0.19	-0.27
Stem diameter	0.1	-0.08
Stem length	0.21	0.14
Nodes per stem	-0.09	0.17
Leaf area	0.02	-0.16

Table 1.3. Correlations between yield and winter survival, shoot per plant, yield per shoot, stem diameter, stem length, nodes per stem, and leaf area in the first production year, 1993.

Parameters	Annual yield	1st harvest	2nd harvest
Winter survival	-0.3	-	-
Shoots per plant	-	0.07	-0.06
Yield per shoot	-	-0.18	0.31
Stem diameter	-	0.06	-0.03
Stem length	-	0.31	0.11
Nodes per stem	-	-0.04	0.18
Leaf area	-	0.23	-0.1

Table 1.4. Correlations between winter survival and TNC, TEG, and LT 50 in the fall of 1992.

Parameters	TNC (%)	TNC/root (g)	TEG	LT 50
Winter survival	0.06	0.23	0.19	-0.04

Table 1.5. Correlations between winter survival and TNC, and TEG in the spring of 1993 after the first winter.

Parameters	TNC (%)	TNC/root (g)	TEG
Winter survival	-0.04	0.25	-0.03

Table 1.6. Correlations between leaf to stem ratio and C.P., ADF, and NDF contents.

Parameters	Fall of 1992	1st harvest, 1993	2nd harvest, 1993
C.P.	0.28	0.23	-0.01
ADF	-0.27	-0.53	-0.19
NDF	-0.08	-0.36	-0.12

Table a.1. Analysis of variance of the effect of plant spacing on yield at the first harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	2141246	0.5766
Plant spacing	5	5066709	0.2164
Linear	1	11107340	0.0695
Quadratic	1	2313014	0.4042
Error	15	3139200	

Table a.2. Analysis of variance of the effect of plant spacing on yield at the second harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	1299979	0.2351
Plant spacing	5	5261805	0.0022
Linear	1	21297179	0.0001
Quadratic	1	1680225	0.1731
Error	15	821252	

Table a.3. Analysis of variance of the effect of plant spacing on yield at the third harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	442766	0.5868
Plant spacing	5	2396717	0.0245
Linear	1	10046860	0.0015
Quadratic	1	93045	0.7139
Error	15	666499	

Table a.4. Analysis of variance of the effect of plant spacing on annual total yield in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	4152633	0.3739
Plant spacing	5	30337310	0.0007
Linear	1	123863243	0.0001
Quadratic	1	239961	0.803
Error	15	3722330	

Table a.5. Analysis of variance of the effect of plant spacing on yield at the first harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	1453514	0.1483
Plant spacing	5	2442812	0.0279
Linear	1	2215869	0.0965
Quadratic	1	1123016	0.2251
Error	15	704833	

Table a.6. Analysis of variance of the effect of plant spacing on yield at the second harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	808204	0.1906
Plant spacing	5	2135898	0.0084
Linear	1	738918	0.2191
Quadratic	1	432953	0.3418
Error	15	449203	

Table a.7. Analysis of variance of the effect of plant spacing on annual total yield in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	3761242	0.0315
Plant spacing	5	6613526	0.0017
Linear	1	5513965	0.0311
Quadratic	1	163288	0.6882
Error	15	975115	

Table a.8. Analysis of variance of the effect of plant spacing on winter survival in the spring of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	17.69	0.3324
Plant spacing	5	673.3	0.0001
Linear	1	2552.5	0.0001
Quadratic	1	714.5	0.0001
Error	15	14.34	

Table a.9. Analysis of variance of the effect of plant spacing on winter survival in the spring of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	1.16	0.7035
Plant spacing	5	144.44	0.0001
Linear	1	503.37	0.0001
Quadratic	1	168.34	0.0001
Error	15	2.44	

Table a.10. Analysis of variance of the effect of plant spacing on shoots per plant in the spring of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	40.63	0.0305
Plant spacing	5	207.73	0.0001
Linear	1	1028.77	0.0001
Quadratic	1	1.76	0.6872
Error	15	10.43	

Table a.11. Analysis of variance of the effect of plant spacing on shoots per plant in the spring of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	17.95	0.2498
Plant spacing	5	713.29	0.0001
Linear	1	3537.92	0.0001
Quadratic	1	23.62	0.1776
Error	15	11.8	

Table a.12. Analysis of variance of the effect of plant spacing on shoots per plant at the first harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	32.06	0.5497
Plant spacing	5	807.19	0.0001
Linear	1	3729.54	0.0001
Quadratic	1	279.01	0.0235
Error	15	43.89	

Table a.13. Analysis of variance of the effect of plant spacing on shoots per plant at the second harvest in the first production year of 1992..

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	29.24	0.4456
Plant spacing	5	1384.47	0.0001
Linear	1	6459.89	0.0001
Quadratic	1	400.2	0.0027
Error	15	31.08	

Table a.14. Analysis of variance of the effect of plant spacing on shoots per plant at the third harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	102.56	0.0662
Plant spacing	5	1149.37	0.0001
Linear	1	5342.11	0.0001
Quadratic	1	296.76	0.0105
Error	15	34.69	

Table a.15. Analysis of variance of the effect of plant spacing on shoots per plant at the first harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	6.5	0.5227
Plant spacing	5	606.91	0.0001
Linear	1	2958.32	0.0001
Quadratic	1	15.62	0.1909
Error	15	8.32	

Table a.16. Analysis of variance of the effect of plant spacing on shoots per plant at the second harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	8	0.3762
Plant spacing	5	803.27	0.0001
Linear	1	3946.25	0.0001
Quadratic	1	41.27	0.2964
Error	15	36.27	

Table a.17. Analysis of variance of the effect of plant spacing on yield per shoot at the first harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.38	0.5272
Plant spacing	5	1.76	0.0252
Linear	1	8.23	0.001
Quadratic	1	0.44	0.3624
Error	15	0.49	

Table a.18. Analysis of variance of the effect of plant spacing on yield per shoot at the second harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.08	0.2498
Plant spacing	5	0.3	0.0033
Linear	1	1.43	0.0001
Quadratic	1	0.03	0.4248
Error	15	0.05	

Table a.19. Analysis of variance of the effect of plant spacing on yield per shoot at the third harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.005	0.779
Plant spacing	5	0.108	0.0012
Linear	1	0.488	0.0001
Quadratic	1	0.037	0.1378
Error	15	0.015	

Table a.20. Analysis of variance of the effect of plant spacing on yield per shoot at the first harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.019	0.4751
Plant spacing	5	0.717	0.0001
Linear	1	3.46	0.0001
Quadratic	1	0.02	0.373
Error	15	0.02	

Table a.21. Analysis of variance of the effect of plant spacing on yield per shoot at the second harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.039	0.0693
Plant spacing	5	0.384	0.0001
Linear	1	1.722	0.0001
Quadratic	1	0.136	0.0064
Error	15	0.014	

Table a.22. Analysis of variance of the effect of plant spacing on stem diameter at the first harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.359	0.0039
Plant spacing	5	0.827	0.0001
Linear	1	3.716	0.0001
Quadratic	1	0.285	0.0338
Error	15	0.052	

Table a.23. Analysis of variance of the effect of plant spacing on stem diameter at the second harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.026	0.3009
Plant spacing	5	0.302	0.019
Linear	1	1.192	0.0014
Quadratic	1	0.048	0.4461
Error	15	0.078	

Table a.24. Analysis of variance of the effect of plant spacing on stem diameter at the third harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.025	0.2322
Plant spacing	5	0.315	0.0001
Linear	1	1.252	0.0001
Quadratic	1	0.176	0.0044
Error	15	0.016	

Table a.25. Analysis of variance of the effect of plant spacing on stem diameter at the first harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.056	0.1835
Plant spacing	5	1.01	0.0001
Linear	1	4.984	0.0001
Quadratic	1	0.042	0.2545
Error	15	0.03	

Table a.26. Analysis of variance of the effect of plant spacing on stem diameter at the second harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.046	0.731
Plant spacing	5	0.431	0.0154
Linear	1	1.946	0.0006
Quadratic	1	0.0003	0.958
Error	15	0.106	

Table a.27. Analysis of variance of the effect of plant spacing on stem length at the first harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	22.327	0.6247
Plant spacing	5	370.485	0.0002
Linear	1	1491.24	0.0001
Quadratic	1	239.07	0.0228
Error	15	37.19	

Table a.28. Analysis of variance of the effect of plant spacing on stem length at the second harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	16.062	0.5563
Plant spacing	5	74.199	0.0324
Linear	1	113.263	0.0398
Quadratic	1	37.097	0.2172
Error	15	22.359	

Table a.29. Analysis of variance of the effect of plant spacing on stem length at the third harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	15.842	0.2798
Plant spacing	5	182.02	0.0001
Linear	1	637.038	0.0001
Quadratic	1	175.365	0.0013
Error	15	11.266	

Table a.30. Analysis of variance of the effect of plant spacing on stem length at the first harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	5.319	0.7851
Plant spacing	5	314.675	0.0001
Linear	1	1379.36	0.0001
Quadratic	1	184.928	0.0042
Error	15	16.319	

Table a.31. Analysis of variance of the effect of plant spacing on stem length at the second harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	30.667	0.0694
Plant spacing	5	210.967	0.0001
Linear	1	983.567	0.0001
Quadratic	1	0.167	0.9016
Error	15	10.567	

Table a.32. Analysis of variance of the effect of plant spacing on nodes per stem at the first harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	2.36	0.0153
Plant spacing	5	6.839	0.0001
Linear	1	27.492	0.0001
Quadratic	1	5.565	0.0042
Error	15	0.491	

Table a.33. Analysis of variance of the effect of plant spacing on nodes per stem at the second harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.349	0.5501
Plant spacing	5	0.742	0.2234
Linear	1	1.406	0.1071
Quadratic	1	0.057	0.7348
Error	15	0.479	

Table a.34. Analysis of variance of the effect of plant spacing on nodes per stem at the third harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.01	0.9949
Plant spacing	5	4.777	0.0002
Linear	1	19.594	0.0001
Quadratic	1	1.058	0.1432
Error	15	0.443	

Table a.35. Analysis of variance of the effect of plant spacing on nodes per stem at the first harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	1	0.197
Plant spacing	5	4.5	0.0008
Linear	1	18.32	0.0001
Quadratic	1	2.145	0.0707
Error	15	0.567	

Table a.36. Analysis of variance of the effect of plant spacing on nodes per stem at the second harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.042	0.8948
Plant spacing	5	2.942	0.0001
Linear	1	14.219	0.0001
Quadratic	1	0.03	0.7086
Error	15	0.208	

Table a.37. Analysis of variance of the effect of plant spacing on leaf area at the first harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	9.31E+08	0.248
Plant spacing	5	4.41E+09	0.0012
Linear	1	7.41E+09	0.0033
Quadratic	1	1.20E+10	0.0005
Error	15	6.09E+08	

Table a.38. Analysis of variance of the effect of plant spacing on leaf area at the second harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	5.91E-08	0.0136
Plant spacing	5	1.20E-08	0.1995
Linear	1	582070185	0.0427
Quadratic	1	357089670	0.1034
Error	15	1.19E-08	

Table a.39. Analysis of variance of the effect of plant spacing on leaf area at the third harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	88600928	0.1778
Plant spacing	5	65606903	0.2848
Linear	1	24247125	0.4852
Quadratic	1	22177272	0.5042
Error	15	47351828	

Table a.40. Analysis of variance of the effect of plant spacing on leaf area at the first harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	1.35E+08	0.5255
Plant spacing	5	4.09E+08	0.0921
Linear	1	1.25E+07	0.7923
Quadratic	1	2.33E+07	0.72
Error	15	1.74E+08	

Table a.41. Analysis of variance of the effect of plant spacing on leaf area at the second harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	2.22E-09	0.3771
Plant spacing	5	7.57E-08	0.8567
Linear	1	17292135	0.9273
Quadratic	1	77	0.9998
Error	15	2.01E-09	

Table a.42. Analysis of variance of the effect of plant spacing on leaf to stem ratio at the first harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.00095	0.8635
Plant spacing	5	0.00816	0.1214
Linear	1	0.0034	0.3627
Quadratic	1	0.023	0.0278
Error	15	0.00388	

Table a.43. Analysis of variance of the effect of plant spacing on leaf to stem ratio at the second harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	3.20E-04	0.946
Plant spacing	5	1.61E-02	0.0027
Linear	1	1.71E-02	0.0217
Quadratic	1	1.79E-02	0.0192
Error	15	2.60E-03	

Table a.44. Analysis of variance of the effect of plant spacing on leaf to stem ratio at the third harvest in the first production year of 1992.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	2.30E-02	0.3309
Plant spacing	5	1.92E-01	0.0005
Linear	1	0.716	0.0001
Quadratic	1	0.223	0.0066
Error	15	2.20E-02	

Table a.45. Analysis of variance of the effect of plant spacing on leaf to stem ratio at the first harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	0.00135	0.66
Plant spacing	5	0.0103	0.0145
Linear	1	0.019	0.0146
Quadratic	1	0.024	0.0071
Error	15	0.00388	

Table a.46. Analysis of variance of the effect of plant spacing on leaf to stem ratio at the the second harvest in the second production year of 1993.

Source of variance	Degrees of freedom	Mean square	P > F
Replicates	3	1.00E-03	0.4484
Plant spacing	5	3.50E-03	0.0443
Linear	1	8.00E-03	0.0178
Quadratic	1	5.00E-03	0.0481
Error	15	1.20E-03	

Table a.47. Analysis of variance of the effect of plant spacing on yield in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	5352281	0.0001
Cultivar (C)	1	403628	0.375
R x C	3	215119	0.7296
Plant spacing (P)	4	7176648	0.0001
Linear	1	489309	0.3295
Quadratic	1	7869063	0.0005
P x C	4	682365	0.27
Error	24	493925	

Table a.48. Analysis of variance of the effect of plant spacing on yield in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	3295117	0.158
Cultivar (C)	1	14171950	0.0088
R x C	3	258877	0.9296
Plant spacing (P)	4	5798204	0.0265
Linear	1	8194587	0.0402
Quadratic	1	14552059	0.008
P x C	4	999104	0.6845
Error	24	1742130	

Table a.49. Analysis of variance of the effect of plant spacing on yield in Algonquin and Vernal at the second harvest in the first production year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	394557	0.5964
Cultivar (C)	1	1852872	0.0957
R x C	3	1475691	0.0932
Plant spacing (P)	4	4427074	0.1505
Linear	1	33031	0.8188
Quadratic	1	4088259	0.0166
P x C	4	84452	0.9669
Error	24	616001	

Table a.50. Analysis of variance of the effect of plant spacing on total annual yield in Algonquin and Vernal in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	3587043	0.2539
Cultivar (C)	1	26274789	0.0034
R x C	3	2670737	0.3774
Plant spacing (P)	4	3058447	0.323
Linear	1	7185173	0.1016
Quadratic	1	3213910	0.2661
P x C	4	1086236	0.7797
Error	24	2478652	

Table a.51. Analysis of variance of the effect of plant spacing on winter survival in Algonquin and Vernal in the spring of 1993 after first winter.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	5.479	0.5822
Cultivar (C)	1	8.01	0.3342
R x C	3	2.903	0.7881
Plant spacing (P)	4	29.959	0.0189
Linear	1	81.314	0.0044
Quadratic	1	21.957	0.1158
P x C	4	3.705	0.7719
Error	24	8.249	

Table a.52. Analysis of variance of the effect of plant spacing on % TNC in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	5.243	0.0623
Cultivar (C)	1	0.144	0.7843
R x C	3	0.889	0.7041
Plant spacing (P)	4	5.258	0.0488
Linear	1	6.616	0.0728
Quadratic	1	12.034	0.0184
P x C	4	3.89	0.1164
Error	24	1.88	

Table a.53. Analysis of variance of the effect of plant spacing on grams TNC per root in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.385	0.0003
Cultivar (C)	1	0.012	0.5943
R x C	3	0.034	0.4907
Plant spacing (P)	4	1.389	0.0001
Linear	1	5.492	0.0001
Quadratic	1	0.024	0.4486
P x C	4	0.016	0.8133
Error	24	0.041	

Table a.54. Analysis of variance of the effect of plant spacing on % TNC in Algonquin and Vernal in the spring of 1993 after first winter.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	11.414	0.0281
Cultivar (C)	1	0.001	0.986
R x C	3	3.48	0.3695
Plant spacing (P)	4	0.463	0.963
Linear	1	1.069	0.567
Quadratic	1	0.177	0.8154
P x C	4	3.682	0.3527
Error	24	3.172	

Table a.55. Analysis of variance of the effect of plant spacing on grams TNC per root in Algonquin and Vernal in spring of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.174	0.0001
Cultivar (C)	1	0.03	0.1631
R x C	3	0.0007	0.9853
Plant spacing (P)	4	0.431	0.0001
Linear	1	1.686	0.0001
Quadratic	1	0.032	0.1471
P x C	4	0.003	0.936
Error	24	0.014	

Table a.56. Analysis of variance of the effect of plant spacing on TEG in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	370.15	0.0018
Cultivar (C)	1	67.08	0.2777
R x C	3	22.24	0.7479
Plant spacing (P)	4	969.76	0.0001
Linear	1	3227.6	0.0001
Quadratic	1	638.79	0.0022
P x C	4	88.2	0.2012
Error	24	54.38	

Table a.57. Analysis of variance of the effect of plant spacing on TEG in Algonquin and Vernal in the spring of 1993 after first winter.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	310.55	0.0003
Cultivar (C)	1	19.18	0.4537
R x C	3	32.88	0.4123
Plant spacing (P)	4	83.3	0.0677
Linear	1	98.8	0.0967
Quadratic	1	135.1	0.0545
P x C	4	24.93	0.5652
Error	24	33.06	

Table a.58. Analysis of variance of the effect of plant spacing on LT 50 in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	4.971.6	0.0455
Cultivar (C)	1	1.60E+00	0.3273
R x C	3	7.30E-01	0.7139
Plant spacing (P)	4	1.04E+01	0.0011
Linear	1	23.28	0.0008
Quadratic	1	2.1	0.2631
P x C	4	2.16E+00	0.2801
Error	24	1.60E+00	

Table a.59. Analysis of variance of the effect of plant spacing on shoots per plant in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	1.57	0.4844
Cultivar (C)	1	5.4	0.102
R x C	3	0.81	0.7311
Plant spacing (P)	4	177.62	0.0001
Linear	1	697.32	0.0001
Quadratic	1	0.13	0.7922
P x C	4	3.48	0.1502
Error	24	1.87	

Table a.60. Analysis of variance of the effect of plant spacing on shoots per plant in Algonquin and Vernal in the spring of 1993 after first winter.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	15.77	0.0332
Cultivar (C)	1	0.68	0.7049
R x C	3	1.09	0.8691
Plant spacing (P)	4	367.67	0.0001
Linear	1	1395.6	0.0001
Quadratic	1	16.14	0.0734
P x C	4	2.68	0.6782
Error	24	4.6	

Table a.61. Analysis of variance of the effect of plant spacing on shoots per plant in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	3.35	0.6442
Cultivar (C)	1	0.73	0.7291
R x C	3	4.23	0.5543
Plant spacing (P)	4	470.79	0.0001
Linear	1	1881.03	0.0001
Quadratic	1	0.61	0.7512
P x C	4	1.33	0.9222
Error	24	5.94	

Table a.62. Analysis of variance of the effect of plant spacing on shoots per plant in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	12.35	0.1089
Cultivar (C)	1	11.13	0.1677
R x C	3	6.66	0.3273
Plant spacing (P)	4	1026.41	0.0001
Linear	1	4056.61	0.0001
Quadratic	1	48.34	0.0067
P x C	4	7.06	0.3042
Error	24	5.5	

Table a.63. Analysis of variance of the effect of plant spacing on yield per shoot in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.156	0.0136
Cultivar (C)	1	0.024	0.4252
R x C	3	0.089	0.0834
Plant spacing (P)	4	1.796	0.0001
Linear	1	6.317	0.0001
Quadratic	1	0.716	0.0002
P x C	4	0.012	0.8429
Error	24	0.036	

Table a.64. Analysis of variance of the effect of plant spacing on yield per shoot in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.04	0.5481
Cultivar (C)	1	0.203	0.0688
R x C	3	0.105	0.1616
Plant spacing (P)	4	3.367	0.0001
Linear	1	13.45	0.0001
Quadratic	1	0.001	0.8869
P x C	4	0.044	0.5435
Error	24	0.056	

Table a.65. Analysis of variance of the effect of plant spacing on yield per shoot in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.039	0.1238
Cultivar (C)	1	0.462	0.1274
R x C	3	0.018	0.4304
Plant spacing (P)	4	0.101	0.0001
Linear	1	3.894	0.0001
Quadratic	1	0.378	0.0001
P x C	4	0.005	0.9038
Error	24	0.019	

Table a.66. Analysis of variance of the effect of plant spacing on stem diameter in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.486	0.0001
Cultivar (C)	1	0.025	0.2741
R x C	3	0.044	0.1117
Plant spacing (P)	4	1.73	0.0001
Linear	1	4.939	0.0001
Quadratic	1	1.565	0.0001
P x C	4	0.041	0.1213
Error	24	0.02	

Table a.67. Analysis of variance of the effect of plant spacing on stem diameter in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.018	0.4733
Cultivar (C)	1	0.00025	0.9143
R x C	3	0.08	0.0232
Plant spacing (P)	4	1.705	0.0001
Linear	1	6.68	0.0001
Quadratic	1	0.085	0.0558
P x C	4	0.02	0.448
Error	24	0.021	

Table a.68. Analysis of variance of the effect of plant spacing on stem diameter in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.135	0.0551
Cultivar (C)	1	0.064	0.2516
R x C	3	0.041	0.4669
Plant spacing (P)	4	1.308	0.0001
Linear	1	4.82	0.0001
Quadratic	1	0.367	0.0097
P x C	4	0.066	0.2575
Error	24	0.046	

Table a.69. Analysis of variance of the effect of plant spacing on stem length in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	236.65	0.0001
Cultivar (C)	1	11.88	0.4292
R x C	3	13.56	0.5397
Plant spacing (P)	4	1037.3	0.0001
Linear	1	2192.52	0.0001
Quadratic	1	1181.58	0.0001
P x C	4	35.93	0.1338
Error	24	18.38	

Table a.70. Analysis of variance of the effect of plant spacing on stem length in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	109.49	0.0016
Cultivar (C)	1	65.03	0.0542
R x C	3	96.49	0.0031
Plant spacing (P)	4	501.9	0.0001
Linear	1	1633.77	0.0001
Quadratic	1	253.98	0.0005
P x C	4	7.4	0.7597
Error	24	15.87	

Table a.71. Analysis of variance of the effect of plant spacing on stem length in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	114.5	0.0007
Cultivar (C)	1	37.44	0.1178
R x C	3	27.82	0.1476
Plant spacing (P)	4	217.03	0.0001
Linear	1	0.0001	0.0001
Quadratic	1	0.0208	0.0208
P x C	4	13.43	0.4553
Error	24	14.23	

Table a.72. Analysis of variance of the effect of plant spacing on nodes per stem in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	5.9	0.0183
Cultivar (C)	1	0.11	0.7854
R x C	3	1.1	0.5277
Plant spacing (P)	4	32.56	0.0001
Linear	1	43.98	0.0001
Quadratic	1	68.47	0.0001
P x C	4	0.44	0.8718
Error	24	1.45	

Table a.73. Analysis of variance of the effect of plant spacing on nodes per stem in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.63	0.3421
Cultivar (C)	1	3.6	0.0165
R x C	3	1.2	0.1124
Plant spacing (P)	4	0.65	0.3365
Linear	1	1.98	0.0679
Quadratic	1	0.4	0.3979
P x C	4	0.1	0.9441
Error	24	0.54	

Table a.74. Analysis of variance of the effect of plant spacing on nodes per stem in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	0.74	0.0868
Cultivar (C)	1	0.63	0.1614
R x C	3	0.48	0.2155
Plant spacing (P)	4	2.86	0.0001
Linear	1	0.0001	0.0001
Quadratic	1	0.0109	0.0109
P x C	4	0.4	0.2803
Error	24	0.3	

Table a.75. Analysis of variance of the effect of plant spacing on leaf area in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	3.56E+08	0.0015
Cultivar (C)	1	4.46E+07	0.358
R x C	3	2.11E+08	0.0168
Plant spacing (P)	4	1.29E+09	0.0001
Linear	1	3238838216	0.0186
Quadratic	1	692937028	0.0011
P x C	4	3.44E+07	0.6142
Error	24	5.08E+07	

Table a.76. Analysis of variance of the effect of plant spacing on leaf area in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	6.68E+08	0.55
Cultivar (C)	1	3.00E+08	0.5748
R x C	3	1.51E+09	0.2088
Plant spacing (P)	4	5.21E+09	0.0024
Linear	1	1.71E+10	0.0003
Quadratic	1	7.10E+08	0.3901
P x C	4	3.39E+08	0.8308
Error	24	9.28E+08	

Table a.77. Analysis of variance of the effect of plant spacing on leaf area in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	2.15E+09	0.0815
Cultivar (C)	1	1.69E+09	0.1717
R x C	3	3.00E+09	0.0302
Plant spacing (P)	4	6.22E+09	0.0005
Linear	1	2.31E+10	0.0001
Quadratic	1	2.43E+08	0.5979
P x C	4	7.67E+08	0.4794
Error	24	8.52E+08	

Table a.78. Analysis of variance of the effect of plant spacing on leaf to stem ratio in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	4.971.6	0.0455
Cultivar (C)	1	1.60E+00	0.3273
R x C	3	7.30E-01	0.7139
Plant spacing (P)	4	1.04E+01	0.0011
Linear	1	23.28	0.0008
Quadratic	1	2.1	0.2631
P x C	4	2.16E+00	0.2801
Error	24	1.60E+00	

Table a.79. Analysis of variance of the effect of plant spacing on leaf to stem ratio in Algonquin and Vernal at the first harvest in the first production year of 1993..

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	4.70E-03	0.0814
Cultivar (C)	1	3.97E-02	0.0001
R x C	3	2.48E-03	0.2861
Plant spacing (P)	4	6.59E-03	0.0207
Linear	1	2.57E-02	0.0011
Quadratic	1	3.55E-04	0.6662
P x C	4	4.46E-04	0.9128
Error	24	1.86E-03	

Table a.80. Analysis of variance of the effect of plant spacing on leaf to stem ratio in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	5.00E-03	0.0631
Cultivar (C)	1	5.80E-02	0.0001
R x C	3	7.00E-03	0.0316
Plant spacing (P)	4	4.00E-03	0.1336
Linear	1	6.00E-03	0.0895
Quadratic	1	7.00E-03	0.0592
P x C	4	1.00E-03	0.4016
Error	24	2.00E-03	

Table a.81. Analysis of variance of the effect of plant spacing on crude protein in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	1.17E+01	0.2653
Cultivar (C)	1	2.12E+00	0.6184
R x C	3	9.00E-01	0.9542
Plant spacing (P)	4	9.36E+00	0.3674
Linear	1	4.53	0.4674
Quadratic	1	0.29	0.8534
P x C	4	2.00E+00	0.9127
Error	24	8.31E+00	

Table a.82. Analysis of variance of the effect of plant spacing on crude protein in Algonquin and Vernal at the first harvest in the first production year of 1993

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	9.09E-01	0.3172
Cultivar (C)	1	7.74E+00	0.0034
R x C	3	1.30E+00	0.1803
Plant spacing (P)	4	2.33E+00	0.0313
Linear	1	2.01E+00	0.1107
Quadratic	1	3.77E+00	0.0326
P x C	4	6.70E-01	0.4721
Error	24	7.33E-01	

Table a.83. Analysis of variance of the effect of plant spacing crude protein in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	3.37E+00	0.148
Cultivar (C)	1	8.19E+00	0.0394
R x C	3	2.10E+00	0.3253
Plant spacing (P)	4	3.59E+00	0.1147
Linear	1	7.85E+00	0.0433
Quadratic	1	1.27E+00	0.3999
P x C	4	6.74E+00	0.0139
Error	24	1.73E+00	

Table a.84. Analysis of variance of the effect of plant spacing on ADF in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	9.29E+01	0.0045
Cultivar (C)	1	3.97E+00	0.6282
R x C	3	2.09E+00	0.9433
Plant spacing (P)	4	6.55E+01	0.013
Linear	1	38.13	0.1414
Quadratic	1	6.11	0.5483
P x C	4	6.07E+00	0.8289
Error	24	1.65E+01	

Table a.85. Analysis of variance of the effect of plant spacing on ADF in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	1.19E+01	0.0254
Cultivar (C)	1	2.24E+01	0.0145
R x C	3	5.00E-01	0.9243
Plant spacing (P)	4	4.96E+00	0.2221
Linear	1	5.57E+00	0.2005
Quadratic	1	2.06E+00	0.4312
P x C	4	4.23E+00	0.2953
Error	24	3.22E+00	

Table a.86. Analysis of variance of the effect of plant spacing ADF in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	3.92E+00	0.4893
Cultivar (C)	1	8.67E+01	0.0003
R x C	3	4.44E+00	0.4351
Plant spacing (P)	4	1.24E+00	0.8986
Linear	1	4.10E-01	0.7696
Quadratic	1	1.72E+00	0.5517
P x C	4	9.73E+00	0.1169
Error	24	4.71E+00	

Table a.87. Analysis of variance of the effect of plant spacing on NDF in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	1.25E+02	0.0049
Cultivar (C)	1	1.04E+01	0.5027
R x C	3	2.96E+00	0.9403
Plant spacing (P)	4	6.25E+01	0.0498
Linear	1	51.9	0.1417
Quadratic	1	28.34	0.2725
P x C	4	2.30E+00	0.9807
Error	24	2.25E+01	

Table a.88. Analysis of variance of the effect of plant spacing on NDF in Algonquin and Vernal at the first harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	2.01E+01	0.0185
Cultivar (C)	1	2.64E+01	0.0299
R x C	3	1.23E+00	0.8623
Plant spacing (P)	4	6.36E+00	0.3041
Linear	1	1.12E+01	0.1456
Quadratic	1	1.43E+00	0.5959
P x C	4	6.49E+00	0.2953
Error	24	4.96E+00	

Table a.89. Analysis of variance of the effect of plant spacing NDF in Algonquin and Vernal at the second harvest in the first production year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	7.40E+00	0.3412
Cultivar (C)	1	8.97E+01	0.0009
R x C	3	4.78E+00	0.5295
Plant spacing (P)	4	1.59E+00	0.9062
Linear	1	1.51E+00	0.6186
Quadratic	1	1.80E-01	0.8688
P x C	4	1.26E+01	0.1283
Error	24	6.32E+00	

Table a.90. Analysis of variance of the effect of plant spacing on yield in Algonquin and Vernal in the fall of establishment year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	4.25E+05	0.7581
Cultivar (C)	1	8.15E+05	0.3983
R x C	3	1.15E+06	0.39
Plant spacing (P)	4	5.68E+06	0.0038
Linear	1	1.74E+07	0.0006
Quadratic	1	4.69E+06	0.0502
P x C	4	8.43E+05	0.6651
Error	24	6.39E+05	

Table a.91. Analysis of variance of the effect of plant spacing on shoots per plant in Algonquin and Vernal in the fall of establishment year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	2.32E+00	0.3327
Cultivar (C)	1	1.00E-01	0.8223
R x C	3	3.18E+00	0.2065
Plant spacing (P)	4	2.95E+02	0.0001
Linear	1	1.17E+03	0.0001
Quadratic	1	5.30E+00	0.1114
P x C	4	9.70E-01	0.7357
Error	24	1.94E+00	

Table a.92. Analysis of variance of the effect of plant spacing on yield per shoot in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	4.40E-01	0.0338
Cultivar (C)	1	2.80E-01	0.1516
R x C	3	4.00E-02	0.8092
Plant spacing (P)	4	3.18E+00	0.0001
Linear	1	12.46	0.0001
Quadratic	1	0.06	0.5139
P x C	4	1.90E-01	0.2491
Error	24	1.30E-01	

Table a.93. Analysis of variance of the effect of plant spacing on stem diameter in Algonquin and Vernal in the fall of establishment year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	1.93E-01	0.1549
Cultivar (C)	1	2.10E-01	0.162
R x C	3	8.00E-03	0.9694
Plant spacing (P)	4	1.43E+00	0.0001
Linear	1	5.59E+00	0.0001
Quadratic	1	3.00E-04	0.9553
P x C	4	6.80E-02	0.6186
Error	24	1.01E-01	

Table a.94. Analysis of variance of the effect of plant spacing on stem length in Algonquin and Vernal in the fall of establishment year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	8.96E+01	0.0466
Cultivar (C)	1	5.93E+00	0.6556
R x C	3	3.19E+01	0.3695
Plant spacing (P)	4	9.78E+01	0.0255
Linear	1	1.50E+02	0.0325
Quadratic	1	6.90E+01	0.1365
P x C	4	4.42E+01	0.2285
Error	24	2.91E+01	

Table a.95. Analysis of variance of the effect of plant spacing on nodes per stem in Algonquin and Vernal in the fall of establishment year of 1992.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	4.20E-01	0.6979
Cultivar (C)	1	3.00E-04	0.9866
R x C	3	3.28E-01	0.7691
Plant spacing (P)	4	2.70E+00	0.0337
Linear	1	10.72	0.0018
Quadratic	1	0.05	0.8141
P x C	4	1.12E+00	0.2994
Error	24	8.70E-01	

Table a.96. Analysis of variance of the effect of plant spacing on leaf area in Algonquin and Vernal in the fall of establishment year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	7.66E+08	0.6429
Cultivar (C)	1	2.56E+09	0.182
R x C	3	1.22E+09	0.4559
Plant spacing (P)	4	3.76E+09	0.0499
Linear	1	1.30E+10	0.005
Quadratic	1	4.64E+06	0.9538
P x C	4	4.39E+08	0.3587
Error	24	1.35E+09	

Table a.97. Analysis of variance of the effect of plant spacing on leaf to stem ratio in Algonquin and Vernal in the fall of establishment year of 1993.

Sources of variance	Degrees of freedom	Mean square	P > F
Replicates (R)	3	3.10E-02	0.0081
Cultivar (C)	1	6.60E-02	0.0033
R x C	3	1.10E-02	0.1725
Plant spacing (P)	4	1.10E-02	0.1544
Linear	1	8.00E-04	0.7226
Quadratic	1	2.32E-02	0.0658
P x C	4	7.00E-03	0.4016
Error	24	6.00E-03	